PRECLINICAL STUDY OF SIDDHA DRUG GANDHAGA PARPAM'S ANTI CANCEROUS, ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

Dissertation submitted to

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In partial fulfilment of the requirements

for the award of the degree of

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BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

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PALAYAMKOTTAI
DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre clinical study of herbo mineral drug *GANDHAGA PARPAM* for its anti-cancerous, anti-oxidant and anti-inflammatory activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Antony Duraichi M.D(s)., Lecturer Gr-II,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVIATIONS

GP	-	GANDHAGA PARPAM
CPCSEA	-	Committee for the purpose of control and supervision of experimental animals.
DC	-	Differential Count
ESR	-	Erythrocyte Sedimentation Rate
FTIR	-	Fourier transform infrared spectroscopy
Hb	-	Haemoglobin
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
Ig E	-	Immunoglobulin E
LDH	-	Lactate Dehydrogenase
MCV	-	Mean Corpuscular Volume
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning electron microscope
CCD_S	-	Charge coupled devices.
SPME	-	Solid phase micro extraction
TCD	-	Thermal conductivity detector
FID	-	Flame Ionization detector
CCD	-	Catalytic combustion detector
LD	-	Low dose
Mg		Milligram
Kg		Kilogram
LD_{50}		Lethal Dose $_{50}$
p.o		peros
ML		Milliliter
%		percentage
R&D		Research and Development

EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose

CONTENTS

TABLE CONTENTS

FIGURE CONTENTS

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Name of the principle investigator : Dr. Suntharalingam Thanaranjan

Title of the Project : Gandhaga parpam for its Anti – inflammatory, Anticancerous, Antioxident activities

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TAMIL NAME	ENGLISH NAME	CHEMICAL NAME
GANTHAGAM	**SULPHUR**	**SULPHUR**

Date : 4.7.18.

Station: Palayamkottai.

4/7/18

Authorised Signature.

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Tamil Name	Botanical Name	Family	Part Used
Oddukkan	*Cleistanthus collinus, (Roxb.)Benth.&Hook.*	Phyllanthaceae	Leaf

Date : 4.7.18.

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1. INTRODUCTION

"When the stem of a flower gets slightly deviated,
The florescence doesn't diminish
When the sugarcane stalk is deviated,
The sweetness flows
When on iron rod is bent,
It controls even on elephant,
But, when a nerve gets bent, what can be done?"

This is old tamil saying, which the siddha healers challenged!

The ultimate purpose of any scientific research is to bring solutions to human problems. The science of contemporary age witnesses science of different turn. Our way of relying on technology and mathematical modeling has turned that attention to things that have little or nothing to do with day to day concerns of the human race. The ancient siddhars speak of numerous remedies solving basic human problems, using everything available in the habitat. This medicinal knowledge remains unsurprised in the entire world. Our current day problems such as cancer and HIV/AIDS seen identical to their descriptions of certain diseases for which they have also prescribed healing methods and treatments. The present research aims to analyze the medicinal utilization of *GANDHAGA PARPAM* and it's medicinal activities like anti-cancerous, anti-oxidant and anti-inflammatory.

Siddha traditional system of medicine is widely being practical in the tamilnadu and the concept pertaining to drug ingredients are from plant (Mooligai), Mineral(Thathu), Metals and Animal(Jeevam) origin.

Cancer (*Yoni putru*) is one of the leading causes of mortality world wide and the burden is increasing day to day. This dreadful disease existed in our society from time immemorial and the way of management including therapeutic aspects has been widely described in classical siddha texts. Selected *GANDHAGA PARPAM* which is quoted in agasthiyar vaithiya vallathy 600 published by CCRS-pub.15.

The objective of the present study is to analyses the *GANDHAGA PARPAM*'S therapeutic activities to be utilized for the treatment of cancer and inflammation. The hypothesis of the research is to prove that. *GANDHAGA PARPAM*'S therapeutic activities like anti-cancerous, anti-inflammatory and anti-oxidant has shown better

results in the cancer and inflammation treatment comparing to some of the standard modern drugs for the same. Hence the present research is a unique one in creating awareness of the siddha herbo mineral drug and paves way for further research in the utilization of *GANDHAGA PARPAM* to cure the modern drug contagious disease.

2. AIM AND OBJECTIVES

AIM

The aim of this present study is to scientifically validate traditional siddha medicine *Ganthaga parpam* for its Anti-cancer, Anti-oxidant, Anti inflammatory activities.

OBJECTIVES

- The main objective of these studies to collect various literatures which includes traditional siddha literatures basic modern aspect of the test drug and the diseased view.
- To prepare the drug according to siddha classical literature illustrations to standardize the drug by appropriate physico-chemical analysis.
- To analyse the drug chemically for reduction of metals, minerals to access the safety profile of the drug by acute and chronic oral toxicity profiles of *Ganthaga parpam* according to OECD guidelines.
- To evaluate the anti-cancer activity of the test drug *Ganthaga parpam* by invivo Dalton's Lymphoma Ascites method.
- To access anti-oxidant activities of the test drug *Ganthaga parpam* by using invitro DPPH free radical scavenging method.
- To enumerate the Anti inflammatory activity of test drug *Ganthaga parpam* by using Carrageenan induced hind paw edema method.
- To evaluate exact bio-chemical content of *Ganthaga parpam* by using various standard protocol.
- To evaluate the mineral content of *Ganthaga parpam* by various instrumental analysis.
- To evaluate the efficacy of the drug *Ganthaga parpam* by various analysis.

3.REVIEW OF THE LITERATURE

3.1. *SULPHUR* – கந்தகம்

3.1.1. GUNAPADAM ASPECT :

SOURCE

A non-metallic element found free in beds of gypsum and in a state of sublimation in regions of extinct volcanoes; also in combination with several ores called pyrites, as sulphates and sulphides of iron, copper, lead, zinc, mercury etc. It is a constituent of various vegetable and animal substances such as albumen etc. It is obtained by roasting, fusion or by sublimation.

More than 98 per cent of the native sulphur produced in the United States at this time comes from deposits in Louisiana and Texas, Nepal, Afghanistan, Kashmir, Burma etc. In India it is found in combination with other metals, minerals, herbal and animal products.

Naturally Occurred as a sublimation product at volcanic fumaroles; a product of the activity of biological micro-organisms; as a result of low oxidation potential and highly acidic chemical reactions in mineral deposits; formed by the decomposition of sulfides, especially pyrite, during mine fires; found in sedimentary rocks.

***Ganthagam* (Sulphur) in siddha medicine**

Siddhar Bhogar classified the metals and minerals into four groups in his book "*Bhogar karasara thurai*" They are,

1. Metals (*Ulogam*)-12
2. Toxins (*Padanam*)-64
3. Minerals (*Karasaram*)-24
4. Hydrochemicals (*Uparasam*)-120

"*Ganthagam"*or Sulphur is one among the *Padaanam*. Sulphur is a crystalline, non-metal used in the preparations like *Rasayanam, mathiri, Mezhugu, Parpam* and *Chenduram* as major ingredients in *Siddha* therapeutics.

In Siddha medicine, it was clinically used as a therapeutic ingredient in Skin care therapies. In solid state, it possesses bitter and astringent.

Types of *Ganthagam:*

There are four types of ganthagam, which has been discussed in Siddha literatures. They are as follows,

1. *Pirappu gandagam,*
2. *Kozhi thalai gandagam,*
3. *Vana Kendhi vaipu*,
4. *Vaipu gandagam,*

Pirappu gandagam

It occurs naturally in mines and rocks and specially this type of ganthagam can be used directly for the medicinal preparation after the definite purification process as mentioned in literatures. Exclusively which is originated in mountain rocks.

Kozhi thalai gandagam

The term suggests that it resembles like a red coloured cock crown. The vaippu ganthagam, Kozhi thalai gandagam & Vana kendhi gandhagam are the by-products of Pirappu gandhagam.

Classification based on Characteristic features

Nellikai gandhagam has widely used in the siddha medicinal formulations. Pagai sarakku for gandhagam is Sembu (Copper). Natpu sarakku for gandhagam is Rasam (mercury).

Traditional literary names for ganthagam

Traditionally ***Sulphur/ganthagam*** is known with other names like *Ghandagam, Kaarizhai Natham, Parai natham, Parai Veerayam, Atheetha prakasam, Beejam, Selvi vindhu, Sakthi, Sakthi peesam, Chenduraa thrrathi, Theviuram, Natham, Narram, Parai natham, Ponnvarni, Rasa sronitham.*

Kozhi thalai kanthagam also named as *Kozhiyin Kudakar, Suga thundam, Sigappu Kenthi, Shanathil Sithi, Yoha sithi, Tharuvon, Sathakkaranthi, Aanathavalli, Karpagam, Porkami* etc.

Ganthagam- Comparison with Mother Nature

மாதர் மகவை வளர்ப்பது போல் உடம்மை

யாதரவாகத் தேற்றி யாக்கையினால் –Verse of Theran Porut panbu nool

The drug ganthagam has compared with the Mother Nature. It is like the diseases could be cleared by the medicines as like how a mother nurtures their child during their childhood caring period.

CHARACTERS

The gandhagam are again classified based on its special characteristic features like colour, odour, shape etc. Here the classical literatures were separated based on its colour.

It is of four types namely

1. White coloured gandhagam, which is ideal for the treating diseases
2. Golden coloured (nellikai gandhagam).
3. Colour of crow i.e shiny black.
4. Red as that of beak of parrot.

(1) The white variety known as roll sulphur is found in sticks about two inches in width and 3 to 5 inches long; the taste is bitter and astringent and the smell is nauseous. It is very brittle; it is somewhat sticky to touch. It being inferior to the yellow variety is preferred for external application.

(2) 'Yellow variety or vitreous or precipitated sulphur or Amlasar gandhaka, occurs in semi-transparent crystals resembling the translucent ripe fruits of the Amalaki. This is employed for internal use in combination with mercury.

(3) The black variety, i.e., Sublimed sulphur (Gandhak-na-Phul) is a purified form of sulphur and is prepared by washing Gandhaga in milk. It is first dissolved in an iron ladle smeared with butter and then gradually poured into a basin of milk.

(4) The red variety is called Rati Hirakasi or Lal gandhak; it occurs in small flat or irregular crystalline pieces of a shining orange-red, purple or brick dust colour. The taste is acrid and bitter. It burns with a faint blue flame and emits the smell of sulphur.

In addition, Gooseberry sulphur(*Nellikkai Gandhagam*) and stick sulphur(*Vaana Gandagam*) have been mentioned in most of the text books of ancient siddhamedicine. Gooseberry sulphur is the one which is often used in medicinal preparations.

SYNONYMS :

Traditionally ***Sulphur/ganthagam*** is known with other names like *Ghandagam, Kaarizhai Natham, Parai natham, Parai Veerayam, Atheetha prakasam, Beejam, Selvi vindhu, Sakthi, Sakthi peesam, Chenduraa thrrathi, Theviuram, Natham, Narram, Parai natham, Ponnvarni, Rasa sronitham.*

Kozhi thalai kanthagam also named as *Kozhiyin Kudakar, Suga thundam, Sigappu Kenthi, Shanathil Sithi, Yoha sithi, Tharuvon, Sathakkaranthi, Aanathavalli, Karpagam, Porkami* etc.

\- *Gunapadam Thathu Jeeva Vaguppu*

கெந்தகத்தின் பேர்தனையே கூறக்கேளு செந்தகோ கெந்தமா தனமுமாகும்
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கனி சத்தி தன் மாதக் குண சலாகுந் தமனியமாம் நாறும்பூ பட்சமாகும்
செந்தியாஞ் செந்தூரத் தாதியாகுஞ் செப்பிய தோர் பேரெல்லாங் கெந்தகமுமாமே

-போகர் நிகண்டு

கந்தி பொன்வர்ணங் காரிழை நாதமாம் அந்திப நாறும்பூ வதி ரச சுரோணிதாம்
தந்திய சத்தி தன் மாதக் குணச்சலாம் பந்திய கெந்தி பரிபாஷை நாமமே''

-சட்டமுனி நிகண்டு

VERNACULAR NAMES

Sans.	:	Gandhaga
Eng.	:	Brimstone, Sublimed Sulphur
Hind.	:	Gandak, Gundhak
Ben.	:	Gandrak, Gandhak, Kush
Mal.	:	Gendagum
Tam.	:	Gandakam
Tel.	:	Gandhagam
Punj.	:	Gandhak, Kibrit, Anwlasar, Gogird
Arab.	:	Kibrika

ORGANOLEPTIC CHARACTORS

Colour	:	Yellow
Potency	:	Hot
Taste	:	Bitter and astringent

ACTIONS

- ❖ Laxative
- ❖ Tonic

- ❖ Antiseptic
- ❖ Alterative
- ❖ Diuretic
- ❖ Insecticide

Sulphur when taken internally and in small doses becomes absorbed and may be excreated through the sweat, milk and urine. It has a specific action on the rectum and increases haemorrhoidal secretions. In large doses it acts as a purgative.

TYPES AND ITS GENERAL PROPERTIES:

***Nellikkai Gandhagam* (Gooseberry Sulphur):**

"நெல்லிக்காய் கெந்த கத்தி னெறி கேளாய் கவுசி
குன்மம்வல்லதாம் வாயு குட்டம் வலிவிடங் கடுங் - கிரந்தி
சொல்லிடுஞ் சுரங்களெல்லாந் தொலைந்திடு மென்று முன்னர்பல்வகை
முனிவர் சித்தர் பகர்ந்தவாக கடங்கள் சொல்லும்"

- பதார்த்த சுடாமணி

"நெல்லிக்காய்க் கந்திக்கு நீள்பதிணென் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறு கரம் பேதி
திடக்கிரக ணீகபம்போந் தேர்."

- குணபாடம் தாது சீவ வகுப்பு

This is considered to useful in the treatment of 18 types of skin diseases, liver enlargement, abdominal distension, eye diseases, chronic venereal diseases, chronic diarrhoeas, gastric ulcer, poisonous bites, fever due to vatha, chronic dysentry etc.

***Vaana Gandhagam* (Stick Sulphur):**

"வாணக் குழாய்க்கந்தி வாசனையைக் கண்டவுடன்
காணக் கிருமி சொறி காணாவாம் - தோணும்
பெருவியா திக்கூட்டம் பேருமத னூலின்
மருவியா முங்கொடியே வாழ்த்து."

"மாதர் மகவை வளர்ப்பதுபோ லேயுடம்பை
யாதரவா கத்தேற்றி யாக்கையினால் - மீதாக
மேவி யடர்நோயின் வெப்பத்தை மாற்றுதலாற்
றேவியுர மென்பதுடல் தேர்."

This sulphur is useful to control the pathogenic micro organisms in the blood. It is also useful in the treatment of chronic joint disorders, scabies, asthma, heart attack, cough, anorectal diseases, leprosy etc.

PURIFICATION OF SULPHUR:

1. The exudate obtained from outer shell of tamarind fruit (210 gm) is collected and placed in a mud pot. Old rice water fermentation, sour butter milk and the juice of mushroom (210 gm each) are also poured in the pot. The pot is covered with a cloth over which 3.5 gm of sulphur is placed and the pot is covered with a lid. The pot is heated for about 6 hours; the purified sulphur settles down.
2. The *kalkam* of Lawsonia inermis is mixed in cow's curd and placed in a mud pot. The mouth of the pot is covered with a cloth. Sulphur is placed over this cloth. The pot is covered with another pot and burried in the ground. The outer pot is subjected to puda with five dung cake. The sulphur which melts and settles down is collected. This procedure is repeated for 7 times.
3. Sulphur is placed in an iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts; this mixture is immersed in inclimed position in cow's milk. This procedure is repeated for 30 times to get purified sulphur. Each times, fresh milk is to be used.
4. Sulphur is melted in the stem juice of plant tree for ten times to get it purified.

TRADITIONAL USES

- Sulphur is given with mercury to treat in almost all diseases.
- Sulphur with Jaggery or cream of Milk, is given to treat diseases like haemorrhoids, porolaps and stricture, also in chronic skin diseases;
- In skin diseases sulphur is used both internally and externally.
- Sulphur and *Yavakshara* (Hordeum vulgare- Barley) mixed with mustard oil is applied in pityriasis, psoriasis, etc.
- In chronic skin diseases a confection of sulphur called Ganthaka rasayana is used as an alterative.it is given with hot water before every meal, in acute leprosy, has been beneficial.

TOXIC SYMPTOMS OF GANDHAGAM

Sulphur, without proper purification, if taken in an exceedingly high dose, or if taken in the medicinal dose for a certain period of time, causes poisoning. It produces the following diseases e.g. skin diseases, loss of semen and beauty,general debility etc.

ANTIDOTE

- Cow's milk with cow's ghee, required quantity to be administered for seven days to detoxify Sulphur poisioning.
- The decoction to prepared from in equal quantity of *Cassia auriculata root, Gynandropsis gynandra root, Zingiber officnale root, Gossypiun herbaceum root, Mesua nagassarium root and Indigofera tinctoria root.*
- The decoction to prepared from equal quantity of *Piper nigrum, Cuminum cyminum and Indigofera tinctoria root.*
- *Nelumbo nucifera* seeds grinded with coconut water and filtered.

OTHER PREPARATION :

1. Ananda Bairavam Mathirai

Dosage	:	1 to 2 pills twice a day.
Adjuvant	:	honey or ginger juice.
Indications	:	fevers and janni

2. Ashta Bairavam

Dosage	:	1 pill twice a day
Adjuvant	:	breast milk, honey, lavanga decoction,
Indications	:	fevers, doshams, vishams.

3. Gandaka Mezhugu

Dosage	:	2 to 5gms, twice a day.
Indications	:	vegu-moothram, sori, sirangu, kushtam,moolam,etc.

4. Gandhaga Sudar Thailam

Dosage	:	1 to 4 drops
Indications	:	*venkushtam (leucoderma), megapun(syphilitic and other chronic ulcers)*

5. ***GANDHAGA PARPAM*** :

Dosage	:	¼ to ½ gms
Adjuvant	:	ghee or butter.
Indications	:	skin diseases, such as sori, themal,meghapadai, kushtam, mathumegam, swasam, kshayam, moolam, bagandaram, etc.

SULPHUR :

3.1.2GEOLOGICAL ASPECT :

Sulphur is one of the most abundant and ubiquitous elements in living things. It is present in organic materials throughout the universe.

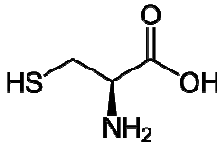
Name	**:**	Sulphur
Symbol	**:**	S
Other name	**:**	Brim stone
Atomic Number	**:**	16
Atomic weight	**:**	32.064+/-0.003
Melting point	**:**	119-120C
Boiling point	**:**	44.601C
Valencies	**:**	2,4,6
Crystal System	**:**	Orthorhombic
Habit	**:**	Crystals common; typically dipyramidal; thick tabular on {001},also massive, in spherical or reniform shapes, incrusting.
Twinning	**:**	Rare on {011}
Cleavage	**:**	{001}, {110} indistinct. Conchoidal to uneven.
Hardness	**:**	1½ - 2½.
Tenacity	**:**	Brittle to slightly sectile.
Specific Gravity	**:**	2.07
Color And Streak	**:**	Yellow to yellowish brown.
Storage	**:**	Store in closed bottle at ambient temperatures
Precautions	**:**	Chronic inhalation of dust can cause irritation of the mucous membrane.

SOURCE:

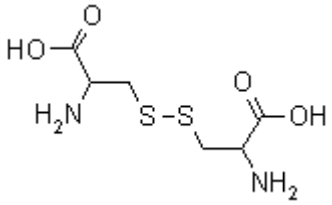
Organic:

Sulphur containing amino acids cystine, cysteine and methionine

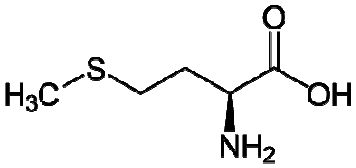
Cysteine: (Amino –thio propionic acid)



Cystine: (Dicysteine)



Methionine: (amino methylthio –n-butyric acid)



Inorganic

Sodium, Pottasium, and Magnesium sulphates.

Occurrence:

Native sulfur occurs in regions of recent volcanic activity where it has been deposited as a direct sublimation product from volcanic gases, has been formed by incomplete oxidation of hydrogen sulfide from volcanic sources,or has resulted from the decomposition of hydrogen sulfide in thermal spring waters. It also occurs in sedimentary sequences that contain sulfates along with organic materials.

In the combined state sulphur occurs as

1.Sulphides and Sulphates

Sulphides: Eg- Copper pyrites($CuFeS_2$)

Mercury Sulphide (HgS) Cinnabar

Sulphates: Eg- Gypsum($CaSO_4 2H_2O$)

Sulphur occur in any organic compounds viz,in animal and vegetable products, onion, garlic, mustard, raddish, hair, many oils, Egg white, protein etc.

2.Sulfonamides

The anti microbial compound containing sulfonamide group (SO_2NH_2) are called sulfonamides. They are effective against a variety of Gram-Positive, Gram-Negative and certain Chlamydia. They are mainly bacteriostatic but occasionally in

very high concentrations particularly in the urinary tract, they may act as bacterial compounds.

Mode of action of sulphur drugs

All higher animals and number of micro-organisms are unable to synthesize certain essential nutrients like vitamins, unsaturated carboxylic acids, certain amino acids etc.P-amino benzoic acid is needed by the enzyme which are sensitive to sulpha drugs.P-amino benzoic acid is needed by the enzyme within those bacteria for the synthesis of folic acid.

Drugs, which inhibit the growth of any micro-organism are termed anti metabolites. Sulpha drugs are anti metabolites for the bacteria, which need P-amino benzoic acid for the synthesis of folic acid. Sulphanilamide acts as an inhibitor of those enzymatic steps, which are involved in the synthesis of folic acid by the bacteria.

This is because the bacterial enzymes are unable to distinguish between the molecule of P-Amino benzoic acid and that of sulphanilamide there being a close structural similarity between the two sulphanilamide thus inhibits the activity of the bacterial enzyme, which is therefore unable to synthesize requisite folic acid essential for living organisms resulting in the death of bacteria. Humans do not synthesize their own folic acid and get it from dietary sources, they are unaffected, therefore by sulpha drugs.

Associations :

It is commonly associated with titanium, vanadium, manganes, coppers, silver, strontium, boron, zinc, zinconium, lead, chromium, arsenic.

Bio- Chemistry

Sulphur a nutritionally essential element is present in all cells of the body, occurring mainly in protein, in the form of sulphur containing amino acids. It is present in certain enzymes, hormones, chondroitin sulphur acid of cartilages and tendons and keratin of hair.

Sulphur in blood:

The total sulphur in blood (plasma) averages about 3.4mgm% this is present in 3 distinct forms

1.In organic sulphate 0.5-1.1mg/100ml

2. Neutral sulphate 1.7-3.5mg/100ml

3. Ethereal sulphate 0.1-1.0mg/100ml

Absorbtion

Sulphur in food is ingested in 2 forms.

1. As inorganic sulphate of sodium, potassium and magnesium
2. As organic sulphate from sulphur containing amino acids, glycoprotein's and chondroition sulphuric acid.

Metabolism

In the liver, sulphur undergoes the following changes

Most of the organic sulphur is oxidized to inorganic sulphates(SO4).But a small portion escapes in oxidation. The un oxidized sulphur is utilized for the formation of sulphur containing substances like insulin, anterior pituitary hormones. And the rest excreted the urine as neutral sulphar.

Morphological features:

Colour, low hardness, brittleness, melts at 113, burns at 270 in air with a blue flame, yielding sulfur dioxide; insoluble in water and unaffected by most acids, soluble in carbon disulfide and some oils.

Importanat Therapeutic Uses

- Urinary tract infections
- Acute bacillary dysentery
- Meningo coccal meningitis
- Haemophillus influenze meningitis
- Chancroid
- Trachoma and inclusion conjunctivitis
- Used as a prophylaxis to prevent attacks of streptococcal tonsillitis in patients who have recovered from rheumatic fever.

Adverse reactions

1. Intolerance

This usually becomes apparent within 1 week but can occur at anytime during the drug administration. The commonest allergic symptoms are drug fever, skin rash usually morbili form in type, eosinophilia, Rash is often accompanied by conjunctivitis.

2. On urinary tract

In the presence of acid urine, the acetylated form of the drug may be precipitated mainly in the collecting tubules and calyces. This causes renal irritation leads to obstruction of the urinary flow and may precipitate cause renal colic. Crystaluria, albuminuria, and haematuria can occur leading to the development of oliguria and anuria.

3. On haemopoietic system

The haemopoietic toxicity includes agranulocytosis, thrombocytopenia causing petechiae, haematuria and epistaxis,rarely aplastic anaemia.

4. Miscellaneous

These include central nervous system disturbances like confusion, depression, ataxia, tinnitus, fatigue and acute psychotic episodes. These disturbances are commonest in children than in rarely peripheral neuritis may occur.

3.1.3.LATERAL RESEARCH

Anti-Cancerous Activity of Rasaoushadhi

Volume : 3 | Issue : 12 | December 2014 • ISSN No 2277 - 8179 Research Paper
Medical Science

ABSTRACT

Rasashastra is a branch of Ayurveda deals about the minerals, metals, precious stones, poisonous herbs having therapeutic property and their processing techniques to prepare medicine which are known as Rasaushadhis. Pharmacopoeia of 'Ayurveda' comprises of drugs derived not only from herbs but also from minerals, metals and animal products. Rasaushadhis occupied superior status among the Ayurvedic Chikitsa due to their high therapeutic potency in eliminating dreadful diseases and also for rejuvenation purposes. Hence, in field of treatment, Ayurvedic Scholars has given the crown of kingdom to Rasa-vaidyas among tri-vidhavaiyas. Nityanada rasa is one of the Rasousahdhi used in the management of Arbuda. In this study we done the physic-chemical analysis and qualitative analysis for the Nityanand rasa tablet. We got the result of physico-chemical analysis of Nityanand rasa.

3.2 *CLEISTANTHUS COLLINUS* – ஒடுக்கன்

3.2.1 GUNAPADAM ASPECT

Synonyms : *Odukku,Oddanthalai,Oduvan,Oduvai,Oduppai,Sittoduvai*

Vernacular names

Eng	:	Karra
Tel	:	Vadisaku
Mal	:	Odukkuhera
Kan	:	Kadise
San	:	Bhallataka-Bijam
Hin	:	Bhilawan

Part used

Leaves, Latex

Organoleptic character

Taste (*Suvai*)	:	Bitter, Punjent (*Kaippu, Karppu*)
Potency (*Thanmai*)	:	Hot(*Veppam*)
Bio-Transformation (*pirivu*)	:	Punjunt (*Karppu*)

Action

Deobstruent

Gentral Characters

It cures Abcess and Lymphnode Enlargement.

Toxic symptoms

- Ingestion of its leaves or a decoction of its leaves causes hypokalaemia and cardiac arrhythemias, metabolic acidosis, hypotension and hypoxia probably due to distal renal tubular acidosis and toxic induced vasodilation.
- Hypokalaemia and acidosis probably also induced rhabdomylosis resulting in myoglobin - uric - renal failure, neuromuscular weakness.

Anti-dote :

Multi dose – Activated charcoal reduces mortality, N-acetyl cystein has been suggested as a possible antidote for Cleistanthus collinus poisoning.

Traditional uses

- The latex of *Odukkan* is applied externally for abcess it subsides day by day.
- *Odukkan* leaves grind well and applied as pattru for externally swelling.
- *Odukkan* leaves juice melted in ganthagam.

Odukkan **in other preparations:**

1. ***Kuthupatatharkku*** **(Stab wounds)**

    - For stab wounds latex of Euphorbia lingularia applied externally along with lemon fomentation on 3rd day
    - *Odduvi ver (Clestanthus collinus root)*
    - *Siru Thambala Ver (Mutilla occidentalis)*
    - *Nannari ver (Hemidesmus indicus)*
    - *Kirambu (Syzygium aromaticum)*
    - *Sirupulladi ver (Desmodiuim triliflorus)*
    - *Sitrelam (Eletterai regains)*
    - *Amanakku vithu (Ricinus communis seed)*
    - *Maruthu (Terminalia arjuna)*
    - *Vembadampattai*

Process of preparation:

All the ingredients are taken in equal amount made into powder then mixed with sesame oil and place the vessel in low till it reach oil consistency. Then cooled and applied on stab wounds.

2. Punnukku Marundhugal:

- *Nilakumizh (Gmelina asiatica)*
- *Oduvan (Clesthanthus collinus bark)*
- *Kambipisin (Gardinia gumnifera)*
- *Vembadampattai*
- *Indu (Mimosa paniculata)*
- *Siru Thambala Ver (Mutilla occidentalis)*
- *Athimathuram (Glyzyrhiza glabra)*

All the ingredients are made into powder and mixed with gingelly oil and place the vessel in low flame till it reach air consistency.

Uses:

For wound due to weapons. For weight loss due to wounds caused by weapon the bark of *Vembadampattai* is smashed and soaked in hot water and use the water to bath.

ODUKKAN (Cleistanthus collinus)

3.2.2 BOTANICAL ASPECT

Taxonomical classification

Kingdom	:	Plantae
Division	:	Angiosperms
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Euphorbiales
Family	:	Euphorbiaceae
Sub Family	:	Phyllanthoideae
Genus	:	Cleistanthus
Species	:	C.collinus

Habitat

Is a plant species first described by roxburgh,with is current name after Bentham and Hooker. It's included in the family phyllanthaceae. The IUCN categorizes this species as vulnerable. No subspecies are listed in the catalogue of life.

Description

Habit

Cleistanthus collinus is a toxic moderate sized deciduous trees,15m high.

Leaves

Leaves simple, alternate, distichous; stipules lateral, 2-3 m long, linear; petiole 3-10 mm long, puberulous to glabrous, slender; lamina 3-11.5 x 1.5-8 cm, elliptic, suborbicular, obovate or acute, apex round, retuse or apiculate, margin entire, glabrous, glaucous beneath, chartaceous; lateral nerves 4-8 pairs, pinnate, slender, prominent, intercostae reticulate, prominent.

Flowers

Flowers unisexual, yellowish-green, in glomerules borne on main leafy branches and short lateral branchlets; the male 3-5 flowered; females upto 3 flowers; bracts 1.5-2 mm long, subulate; male flowers: pedicels 1-2 mm long, puberulous, calyx tube shortly obconic, lobes 5, occasionally 6, 3-4 x 1-2 mm, triangular-oblong or lanceolate-oblong, adpressed fulvous pilose outside; petals 5, linear; disc annular, shallowly lobed, glabrous; staminal column ca.1.5 mm; anthers oblong; pistillode ovoid; female flowers: pedicel 0.5 mm long, grey-pilose; calyx tube obconic, ca.1 mm

long; sepals 4-5 x 1-3 mm, triangular-lanceolate, scattered grey-pilose outside; petals ca. 2 x 1 mm, subulate; disc shortly cupular-annular; ovary superior, 2-2.5 x 2-3 mm, subglobose, glabrous; styles 3-4 mm long, almost free or basal column ca.1.5 mm long, shortly to deeply bifid above, recurved.

Bark

Bark dark brown, nearly black, rough, flaking off in rounded thick scales, blaze red; branchlets, terete.

Fruits and seed

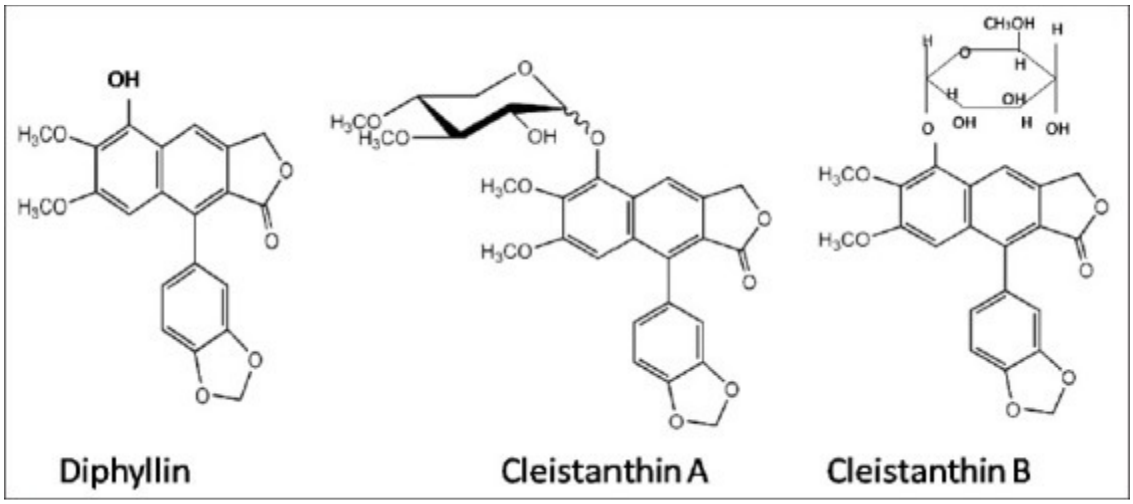
Fruit a capsule, 18-22 x 17-22 mm, subglobose or wide oblong, truncate at apex, shallowly 3 lobed and 3-angled, glossy, glabrous, black or dark brown when dry; seeds 3, globose, black; pedicels 0.5-1.5 mm long.

Chemical constituents

It contains Clestianthin-A, Clestianthin-B, Collinusin, Oduvin-Gulucoside, Sapponin, Tannin

Toxic constituents

Analyses of extracts from the plant, including the leaves, reveal a complex group of compounds. The toxic active principles in the leaves are arylnaphthalene lignan lactones—Diphyllin and its glycoside derivatives Cleistanthin A and Cleistanthin B; and Collinusin Diphyllin, Cleistanthin A and B were collectively known as “Oduvin” in the past. In addition, the lignans Cleistanthin C, Cleistanthin D and Cleistanone, are present.The toxicity of the plant has been attributed primarily to Cleistanthin A and B.



These toxic principles, the arylnaphthalene lignans, are detected in acetone extracts but, are not seen or are present in minimal amounts in aqueous extracts.On spectroscopic analysis of aqueous extracts of fresh *C. collinus* leaves, the other major phytoconstituents detected are 3-O-methyl-d-glucose, benzenetriol (pyrogallic acid), 1.6- anhydro-â-D-glucopyranose (levoglucosan), heptacosane, 2-hydroxy-7-methoxy-4,5-diphenyl-5 hindeno [1.2-d] pyrimidine and eicosane.

Therapeutic uses

- The plant have anti-septic, anti-fungal, insecticidal and larvicidal property
- The leaf extract act against the pathogens like methicillin resistant, staphylococcus aureus (MRSA), enterococcus and candida species. Mostly these pathogens are the causative agents for skin infections and urinary tract infections.

3.2.3 LATERAL RESEARCH:

EVALUATION OF ANTI-CANCEROUS ACTIVITY AND ANTI OXIDANT EFFECT OF CLEISTRANTHUS COLLINUS LEAF EXTRACT IN RATS

ABSTRACT:

Aqueous, methanol and ethyl acetate crude extracts and fractions were prepared using Cleistanthus collinus Roxb. dry leaf powder. All the extracts and fractions were subjected to in vitro cytotoxic analysis using mouse 3T3- L1 pre-adipocytes cell line. Rate of cell proliferation was calculated to determine the anti-proliferative activity. Aqueous, methanol and ethyl acetate extracts (≥100 µg/ml) significantly control cell proliferation at 48 hr incubation. However, fractions exhibited higher level of toxicity and affect cell growth even at 50 µg/ml concentration within 28 hr incubation. Fractions obtained from methanol extract showed cytotoxic effect about 43-76 % at 50-250 µg/ml at 48 hr incubation (Concentration necessary to inhibit cell growth at 50 % is ~75 µg/ml). Followed by, ethyl acetate fraction exhibited 23-59 % of anti-proliferative activity (Concentration necessary to inhibit cell growth at 50 % is ~180 µg/ml). It may be concluded that promising fractions of C. collinus with higher toxicity level could be exploited for pharmacological purposes.

3.3 DISEASE REVIEW

3.3.1 SIDDHA ASPECT

புற்று வரும் வழி (Etiology)

ஊரை பதனழிந்த உணவுகளுண்ணல் வரைபடு புலால் மீன் வழக்கத் துண்ணல்
புணர்ச்சி மிகுதலின் வெப்ப மீறுதல் வெப்ப நிலைகள் வேறுபடுபவரை
அடிக்கடி புணர்தல் ஒழுக்கு நோயுறல் உடலழக்கேலுதல் அணுக்கள் புகுதல்
எனு மிவை பிறவும் பலவகைப்பட்ட புற்று நோய்க் கடிப்படையாகும்.

- மான்முருகியம் என்றும் தமிழ் மருத்துவநூல்

Contaminated foods (fish, mutton), more sexual contact, increased *Pitham*, other climate sexual partner, *vellainoi*, other foreign infectious organism,etc.

புற்றின் முற்குறி (Premonitary symptoms)

புற்றுத் தோன்றிடந் தினவு தோன்றல் அவ்விடம் புரைபட்டெனத் தோன்றிடுதல்
தசைகழிந் தகவல் தளர்ச்சி தோன்றல் உடல்நிலை வேறுபட் டென்னத்தோன்றல்
புற்றும் உறுப்பின் தொழில் குறைந்திடுதல் ஆற்றல் கெடுதல் உறுப்பு நோதல்
சிவத்தல் கடுத்தல் குத்தல் பிறவும் புற்றுநோயின் முன்னமாகும்

Itching, patches, discomfort of the body, loss of energy, pain, redness, swelling and etc.

புற்றின் குறிகுணம் (Sign and symptoms of cancer)

சிறுக சிறுகச் சேவற் பூப்போல கூர்ந்து திரண்டு மழி தசை வளர்தல்
பிணியுற் றிடமும் அயலும் நரம்பும் மிகவும் நோதல் தசையழிந் திடுதல்
நரம்பு முதலிய அரிக்கப்படுதல் குருதியும் பழுப்பு தோன்றல் ஒழுகல்
உடம்பு மெலிதல் தடுமாற் றயர்ச்சி எனுமிவை பிறவும் புற்றின் குறியே

Muscle mass is appear like cock comb, pain present in whole affected part of body, wasting of the muscle and nerves, bleeding of blood and brown colour fluid, body weight is decreased, etc.

புரைதோன்றுதலும் தசை திறைத் திடலும் உட்சதை திரளலும் குருதியொழுகலும் எளிதிற் றீராப் புண் தோன்றிடலும் குருக்கள் போலத் தசைமேற்றொன்றலும் உணவு றாமை தொடர்ந்த தோன்றலும் அடிக்கடி யிருமல் குரலடை தோன்றலும் புள்ளிகள் தோன்றலும் நிறம் வேறுபடுதலும் நீங்காத் தளர்ச்சி யயர்ச்சி தோன்றலும் குடற்பிணி தொடர்ந்த தோன்றலும் பிறவும் புற்றுநோய்க்கும் முன்னமாதலின் முற்பட வுணர்ந்து போதல் மாமே

Patches appear the wound, hardness of muscle, loss of appetite, recurrent cough, loss of voice, malaise, dots appear, colour changes, intestinal diseases and etc.

அல்குல்புற்றின் குணம் (Sign and symptoms of Cervical cancer)

அல்குல் வாயினு மகந்து மருகினும் சேவற்பூப்போல் அழிசதை வளர்தல் நோதல் குத்தல் சீழ்மிக வொழுகல் குருதி வெளிப்படல் தசைநரம் பழிதல் புரைபடல் எனுமிவை பெண்குறிப் புற்றின் குறியென மொழிப நெறிபறி புலவர்

தீரும், தீரா, நோய்க்குறி (Prognosis)

புற்று நோயனைத்தும் பொருந்தப் பேணின் அரிதிற் றீர்வன வாகுமென்ப அறுவையிற் சிற்சில அகல்வது மாகும் மிக்க தளர்ச்சி மெலிவு நீர் வேட்கை பொறிகளின் ஆற்றலழிதல் மயக்கம் காய்ச்சல் தோன்றல் விக்கலெடுத்தல் உணவு செல்லாமை உண்டத றாமை எனுமிவை பிறவுந் தீராக் குறியும்

If select proper treatment to cancer will cure the cancer, surgery may prevent the cancer. But malasise, loss weight, thirst, lack of energy, dizziness, fever and above all the symptoms when appear to patent, will not relive from cancer.

3.3.2 MODERN ASPECT

INTRODUCTION

Cervical cancer starts in a woman's cervix, which is the lower, narrow part of the uterus. The uterus holds the growing foetus during pregnancy. The cervix connects the lower part of the uterus to the vagina and, with the vagina, forms the birth canal.

Cervical cancer begins when healthy cells on the surface of the cervix change and grow out of control, forming a mass called a tumour. A tumour can be malignant or benign. A cancerous tumour is malignant, meaning it can spread to other parts of the body. A benign tumour means the tumour will not spread.

At first, the changes in a cell are abnormal, not cancerous. Researchers believe, however, that some of these abnormal changes are the first step in a series of slow changes that can lead to cancer. Some of the abnormal cells go away without treatment, but others can become cancerous. This phase of the disease is called dysplasia, which is an abnormal growth of cells. The abnormal cells, sometimes called precancerous tissue, need to be removed to keep cancer from developing. Often, the precancerous tissue can be removed or destroyed without harming healthy tissue, but in some cases, a hysterectomy is needed to prevent cervical cancer. A hysterectomy is the removal of the uterus and cervix.

Treatment of a lesion, which is a precancerous area, depends on the following factors:

- The size of the lesion and the type of changes that have occurred in the cells
- If the woman wants to have children in the future
- The woman's age
- The woman's general health
- The preference of the woman and her doctor

If the precancerous cells change into cancer cells and spread deeper into the cervix or to other tissues and organs, then the disease is called cervical cancer.

There are 2 main types of cervical cancer, named for the type of cell where the cancer started. Other types of cervical cancer are rare.

- Squamous cell carcinoma makes up about 80% to 90% of all cervical cancers. These cancers arise in the cells on the outer surface covering of the cervix.

- Adenocarcinoma makes up 10% to 20% of all cervical cancers. These cancers arise in the glandular cells that line the lower birth canal.

The squamous and glandular cells meet at the opening of the cervix at the "squamocolumnar junction," which is the site at which most cervical cancers arise.

In medical research, the most famous cell line known as HeLa was developed from cervical cancer cells of a woman named Henrietta Lacks.

SIGNS AND SYMPTOMS

When present, common symptoms of cervical cancer may include:

- **Vaginal bleeding:** This includes bleeding between periods, after sexual intercourse or post-menopausal bleeding.
- **Unusual vaginal discharge**: A watery, pink or foul-smelling discharge is common.
- **Pelvic pain:** Pain during intercourse or at other times may be a sign of abnormal changes to the cervix, or less serious conditions.

All of these cervical cancer symptoms should be discussed with your doctor.

SIGNS OF ADVANCED STAGES OF CERVICAL CANCER

Cervical cancer may spread (metastasize) within the pelvis, to the lymph nodes or elsewhere in the body. Signs of advanced cervical cancer include:

- Weight loss
- Fatigue
- Back pain
- Leg pain or swelling
- Involuntary urination
- Bone fractures

CAUSES

Cervical cancer begins with abnormal changes in the cervical tissue. The risk of developing these abnormal changes is associated with infection with human papillomavirus (HPV). In addition, early sexual contact, multiple sexual partners, and taking oral contraceptives (birth control pills) increase the risk of cervical cancer because they lead to greater exposure to HPV.

HUMAN PAPILLOMA VIRUS

Forms of HPV, a virus whose different types cause skin warts, genital warts, and other abnormal skin disorders, have been shown to lead to many of the changes in cervical cells that may eventually lead to cancer. Certain types of HPV have also been linked to cancers involving the vulva, vagina, penis, anus, tongue, and tonsils. Genetic material that comes from certain forms of HPV (high-risk subtypes) has been found in cervical tissues that show cancerous or precancerous changes.

In addition, women who have been diagnosed with HPV are more likely to develop a cervical cancer.

SEXUAL CONTACT

Girls who begin sexual activity before age 16 or within a year of starting their menstrual periods are at high risk of developing cervical cancer.

SMOKING

Cigarette smoking is another risk factor for the development of cervical cancer. The chemicals in cigarette smoke interact with the cells of the cervix, causing precancerous changes that may over time progress to cancer. The risk of cervical cancer in cigarette smokers is two to five times that of the general population.

ORAL CONTRACEPTIVES PILL

Oral contraceptives ("the pill"), especially if taken longer than five years, may increase the risk for cervical cancer because they reduce the use of condoms.

MULTIPLE PREGNANCIES

Having many pregnancies is associated with an increased risk of cervical cancer. Among HPV-infected women, those who have had seven or more full-term pregnancies have around four times the risk of cancer compared with women with no pregnancies, and two to three times the risk of women who have had one or two full-term pregnancies.

CHRONIC CERVICITIES

It is the chronic or persistent inflammation of the cervix, which is the lower portion of the uterus. It may be caused by a number of factors, chronic bacterial infections due to staphylococcus, streptococcus, E.coli, anaerobic bacteria, chemical

or physical irritations, injurys to the vagina, sexually transmitted infection(STIs) such as due to Neisseria gonorrhoeae bacterium, and allergies.

DIAGNOSIS

SCREENING

Cervical cancer that is detected early is more likely to be treated successfully. Most guidelines suggest that women begin screening for cervical cancer and precancerous changes at age 21.

SCREENING TESTS INCLUDE

Pap test**:** During a Pap test, your doctor scrapes and brushes cells from your cervix, which are then examined in a lab for abnormalities.A Pap test can detect abnormal cells in the cervix, including cancer cells and cells that show changes that increase the risk of cervical cancer.

HPV DNA test: The HPV DNA test involves testing cells collected from the cervix for infection with any of the types of HPV that are most likely to lead to cervical cancer. This test may be an option for women age 30 and older, or for younger women with an abnormal Pap test.

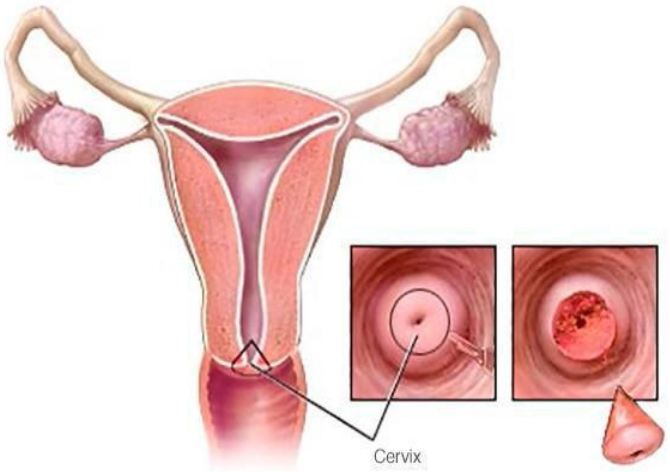


Figure 1: Cervical cancer affected area

CONE BIOPSY

If cervical cancer is suspected, your doctor is likely to start with a thorough examination of your cervix. A special magnifying instrument (colposcope) is used to check for a bnormal cells.

During the colposcopic examination, your doctor is likely to take a sample of cervical cells (biopsy) for laboratory testing. To obtain tissue, your doctor may use:

- Punch biopsy**,** which involves using a sharp tool to pinch off small samples of cervical tissue.
- Endocervical curettage**,** which uses a small, spoon-shaped instrument (curet)
- A thin brush to scrape a tissue sample from the cervix.
- If the punch biopsy or endocervical curettage is worrisome, your doctor may perform one of the following tests:
- Electrical wire loop, which uses a thin, low-voltage electrical wire to obtain a small tissue sample. Generally this is done under local anesthesia in the office.
- Cone biopsy, which is a procedure that allows your doctor to obtain deeper layers of cervical cells for laboratory testing. A cone biopsy may be done in a hospital under general anesthesia.

STAGING

If your doctor determines that you have cervical cancer, you'll have further tests to determine the extent (stage) of your cancer. Your cancer's stage is a key factor in deciding on your treatment.

STAGING EXAMS INCLUDE

- Imaging tests: Tests such as X-rays, CT scans, magnetic resonance imaging (MRI) and positron emission tomography (PET) help your doctor determine whether your cancer has spread beyond your cervix.
- Visual examination of your bladder and rectum: Your doctor may use special scopes to see inside your bladder and rectum.

STAGES OF CERVICAL CANCER INCLUDE

- Stage I: Cancer is confined to the cervix.
- Stage II: Cancer is present in the cervix and upper portion of the vagina.

- Stage III: Cancer has moved to the lower portion of the vagina or internally to the pelvic side wall.
- Stage IV: Cancer has spread to nearby organs, such as the bladder or rectum, or it has spread to other areas of the body, such as the lungs, liver or bones.

CANCER SUBTYPES

Histologic subtypes of invasive cervical carcinoma include the following: Though squamous cell carcinoma is the cervical cancer with the most incidence, the incidence of adenocarcinoma of the cervix has been increasing in recent decades.

- Squamous cell carcinoma (about 80-85%)
- Adenocarcinoma (about 15% of cervical cancers)
- Adenosquamous carcinoma
- Small cell carcinoma
- Neuroendocrine tumour
- Glassy cell carcinoma
- Villoglandular adenocarcinoma

Noncarcinoma malignancies which can rarely occur in the cervix include melanoma and lymphoma. The FIGO stage does not incorporate lymph node involvement in contrast to the TNM staging for most other cancers.

For cases treated surgically, information obtained from the pathologist can be used in assigning a separate pathologic stage, but is not to replace the original clinical stage.

PREVENTION

Cervical cancer can often be prevented by having regular screenings to find any precancers and treat them. Preventing precancers means controlling possible risk factors, such as:

- Delaying first sexual intercourse until the late teens or older
- Limiting the number of sex partners
- Avoiding sexual intercourse with people who have had many partners
- Avoiding sexual intercourse with people who are obviously infected with genital warts or show other symptoms
- Quitting smoking

The HPV vaccine helps prevent cervical cancer caused by HPV (see Risk Factors). Gardasil 9 is available in the United States for preventing infection from HPV-16, HPV-18, and 5 other types of HPV linked with cancer. There were 2 other vaccines previously available in the United States: Cervarix and the original Gardasil. Both of these are no longer available in the United States. However, these vaccines may be in use outside of the United States.

To help prevent cervical cancer, ASCO recommends that girls receive HPV vaccination. Talk with a health care provider about the appropriate schedule for vaccination because it may vary based on many factors, including age and vaccine availability. Learn more about HPV vaccination and ASCO's recommendations for preventing cervical cancer.

Screening is used to look for cancer or abnormalities that may become cancerous before you have any symptoms or signs. Scientists have developed, and continue to develop, tests that can be used to screen a person for specific types of cancer before signs or symptoms appear. The overall goals of cancer screening are to:

Reduce the number of people who die from the cancer, or completely eliminate deaths from cancer.

Reduce the number of people who develop the cancer.

BARRIER PROTECTION

Barrier protection and/or spermicidal gel use during sexual intercourse decreases cancer risk. Condoms offer protection against cervical cancer. Evidence on whether condoms protect against HPV infection is mixed, but they may protect against genital warts and the precursors to cervical cancer. They also provide protection against other STIs, such as HIV and Chlamydia, which are associated with greater risks of developing cervical cancer.

Condoms may also be useful in treating potentially precancerous changes in the cervix. Exposure to semen appears to increase the risk of precancerous changes (CIN 3), and use of condoms helps to cause these changes to regress and helps clear HPV. One study suggests that prostaglandin in semen may fuel the growth of cervical and uterine tumors and that affected women may benefit from the use of condoms. Abstinence also prevents HPV infection.

VACCINATION

HPV vaccines are vaccines that protect against infection with human papillomaviruses (HPV). HPV is a group of more than 200 related viruses, of which more than 40 are spread through direct sexual contact. Among these, several HPV types cause genital warts, and about a dozen HPV types can cause certain types of cancer cervical, anal, oropharyngeal, penile, vulvar and vaginal.

The Food and Drug Administration (FDA) has approved three vaccines that prevent infection with disease-causing HPV types: Gardasil®, Gardasil® 9, and Cervarix®. All three vaccines prevent infection with HPV types 16 and 18, two high-risk HPVs that cause about 70% of cervical cancers and an even higher percentage of some of the other HPV-caused cancers. Gardasil also prevents infection with HPV types 6 and 11, which cause 90% of genital warts. Gardasil 9 prevents infection with the same four HPV types plus five additional cancer-causing types (31, 33, 45, 52, and 58).

Gardasil 9 is the only HPV vaccine available for use in the United States. Cervarix and Gardasil are still used in other countries.

NUTRITION

Vitamin A is associated with a lower risk as are vitamin B12, vitamin C, vitamin E, and beta-carotene.

BRACHYTHERAPY FOR CERVICAL CANCER

Larger early-stage tumors (IB2 and IIA more than 4 cm) may be treated with radiation therapy and cisplatin-based chemotherapy, hysterectomy (which then usually requires adjuvant radiation therapy), or cisplatin chemotherapy followed by hysterectomy. When cisplatin is present, it is thought to be the most active single agent in periodic diseases.

Advanced-stage tumors (IIB-IVA) are treated with radiation therapy and cisplatin-based chemotherapy. On June 15, 2006, the US Food and Drug Administration approved the use of a combination of two chemotherapy drugs, hycamtin and cisplatin, for women with late-stage (IVB) cervical cancer treatment. Combination treatment has significant risk of neutropenia, anemia, and thrombocytopenia side effects.

For surgery to be curative, the entire cancer must be removed with no cancer found at the margins of the removed tissue on examination under a microscope. This procedure is known as exenteration.

PROGNOSIS

Prognosis depends on the stage of the cancer. The chance of a survival rate around 100% is high for women with microscopic forms of cervical cancer. With treatment, the five-year relative survival rate for the earliest stage of invasive cervical cancer is 92%, and the overall (all stages combined) five-year survival rate is about 72%. These statistics may be improved when applied to women newly diagnosed, bearing in mind that these outcomes may be partly based on the state of treatment five years ago when the women studied were first diagnosed. With treatment, 80 to 90% of women with stage I cancer and 60 to 75% of those with stage II cancer are alive 5 years after diagnosis. Survival rates decrease to 30 to 40% for women with stage III cancer and 15% or fewer of those with stage IV cancer 5 years after diagnosis.

According to the International Federation of Gynecology and Obstetrics, survival improves when radiotherapy is combined with cisplatin-based chemotherapy.

As the cancer metastasizes to other parts of the body, prognosis drops dramatically because treatment of local lesions is generally more effective than whole-body treatments such as chemotherapy.

Interval evaluation of the woman after therapy is imperative. Recurrent cervical cancer detected at its earliest stages might be successfully treated with surgery, radiation, chemotherapy, or a combination of the three. About 35% of women with invasive cervical cancer have persistent or recurrent disease after treatment.

Average years of potential life lost from cervical cancer are 25.3. Around 4,600 women were projected to die in 2001 in the US of cervical cancer, and the annual incidence was 13,000 in 2002 in the US, as calculated by SEER. Thus, the ratio of deaths to incidence is about 35.4%.

Regular screening has meant that precancerous changes and early-stage cervical cancers have been detected and treated early. Figures suggest that cervical screening is saving 5,000 lives each year in the UK by preventing cervical cancer. About 1,000 women per year die of cervical cancer in the UK. All of the Nordic countries have cervical cancer-screening programs in place.

EPIDEMIOLOGY

AUSTRALIA

Cervical cancer was the 14th most commonly diagnosed cancer among females in Australia in 2013. In 2017, it is estimated that it will remain the 14th most commonly diagnosed cancer among females.

In 2013, there were 813 new cases of cervical cancer diagnosed in Australia. In 2018, it is estimated that 930 new cases of cervical cancer will be diagnosed in Australia.

In 2013, the age–standardised incidence rate was 6.8 cases per 100,000 females. In 2017, it is estimated that the age–standardised incidence rate will be 7.1 cases per 100,000 females. The incidence rate for cervical cancer is expected to be highest for age group 35–39, followed by age groups 40–44 and 85+ (Figure 1).

The number of new cases of cervical cancer diagnosed decreased from 965 in 1982 to 813 in 2013. Over the same period, the age–standardised incidence rate decreased from 14 cases per 100,000 females in 1982 to 6.8 cases per 100,000 females in 2013.

WORLD WIDE

Worldwide, cervical cancer was the fourth most common cancer among females in 2012. There were an estimated 528 000 new cases of cervical cancer, of which around 85% occurred in less developed regions. Around 266 000 females died of cervical cancer, accounting for 7.5% of all female cancer deaths. About 87% of cervical cancer deaths 1 occurred in the less developed regions.

UNITED STATES

An estimated 12,900 new cervical cancers and 4,100 cervical cancer deaths will occur in the United States in 2015. In the United States, it is the eight-most common cancer of women. The median age at diagnosis is 48. Hispanic women are significantly more likely to be diagnosed with cervical cancer than the general population. In 1998, about 12,800 women were diagnosed in the US and about 4,800 died. In 2014, an estimated 12,360 new cases were expected to be diagnosed, and about 4,020 were expected to die of cervical cancer. Among cancers of the female reproductive tract it is less common than endometrial cancer and ovarian cancer. The rates of new cases in the United States was 7 per 100,000 women in 2004. Cervical

cancer deaths decreased by approximately 74% in the last 50 years, largely due to widespread Pap smear screening. The annual direct medical cost of cervical cancer prevention and treatment prior to introduction of the HPV vaccine was estimated at $6 billion.

EUROPEAN UNION

In the European Union, about 34,000 new cases per year and over 16,000 deaths due to cervical cancer occurred in 2004.

UNITED KINGDOM

Incidence rates for cervical cancer are projected to rise by 43% in the UK between 2014 and 2035, to 17 cases per 100,000 females by 2035. 1 in 135 women will be diagnosed with cervical cancer during their lifetime. Cervical cancer in England is more common in females living in the most deprived areas.

CANADA

In Canada, an estimated 1,300 women will have been diagnosed with cervical cancer in 2008 and 380 will have died.

INDIA

According to a report 'Call for Action: Expanding cancer care for women in India, 2017', cancer among women in India is estimated at 0.7 million. However, the real incidence is much more between 1 and 1.4 million per year as many cases go undiagnosed or unreported.

3.4. PHARMACEUTICAL REVIEW

PARPAM (CALX)

Definition:

Parpam is equivalent to calx, which is prepared by the process of calcination. The correct Tamil translation would be "*Neeru*" which would mean an ash. "*Saambal*" is another word equivalent to an ash or calx.

Metals, *uparasas* or *paashanas* are made into white powder by the process of *pudam*, burning, frying, blowing and by grinding them with juices, *ceyaneer* etc.

பற்ப மகிமை

வீரத்து மிக்கவை பற்பங்களே – பரி
காரத்து மிக்கவை பற்பங்களே
பாருக்குள் மானிடர் நோய்போக – வரு
பண்டிதருக்கெல்லா மாமோகம்
வீரகடாரி – பிணிக்கொரு
பாரகுடாரி – விசைபெ
தீரதடாரி – வினையுடு
சூரிக்குழு நேரொத்தது
மேருக்கினை பாரப்புறம்
தேரன் தரு

பற்ப மருந்துகள்	:	முழுவன்மையும்
செந்தூரங்கள்	:	முக்கால்வன்மையும்
ஏனையவை	:	இதற்கு குறைந்த வன்மையுடையன

Equipment required:

- Mortar and pestle.
- Vessels and spoons to handle liquids.
- Long ribbons of tough cloth and fine clay.
- Pairs of shallow earthen discs of identical dimensions.
- Cow dung cakes, sufficient numbers and well dried.
- Fine cloth pieces for filtering juices and decoction.
- Spatulae for handling powders.

General method of preparation:

The drugs are ground according to the particular recipe, with other drugs, juices or decoctions, and the resultant mass is made into small, thin circular cakes and dried .When they are well dried, they are taken for calcination.

The material ready for calcination, is put into an earthen disc described earlier, and covered by inverting another disc and sealing the rim with the cloth ribbon one side of which is smeared with wet clay. This makes a capsule type crucible. When the seal is dry, the capsule is placed in the kiln for calcination.

The kiln or "*pudam*" as it is called in Tamil, is made by digging a pit of appropriate dimensions in the soil, and filling it with the recommended number of cowdung cakes, which is the fuel. It is better that the interior of the pit is lined with bricks, so that the pit could be used repeatedly.

Seventy five percent of the recommended numbers of dung cakes are arranged in the pit and then the capsule is placed in the centre. The rest of the dung cakes are arranged above this and the top is somewhat dome shaped. When some burning charcoal pieces are placed on the dome, the dung cakes below them catch fire and the fire spreads all around in a uniform manner.

The kiln will burn for a long time, until all the dung cakes are burnt and converted into ashes. When the kiln cools down, the ashes are very carefully removed and the capsule is taken out without damaging the seat. The exterior of the capsule is thoroughly brushed to remove the ashes and the seal is scraped off and removed.

The contents of the capsule are recovered, and the remnants that adhere to the walls are gathered by great scraping and brushing.

For the complete transformation of the material into "*parpam*" state, the process of grinding, drying and calcination may have to be repeated several times or atleast as many times as directed in the recipe. However, the calcination is repeated until a satisfactory product is obtained. But in those instances where the number of calcinations is definitely indicated, the process should be repeated accordingly, even if a satisfactory "*parpam*" is obtained within a few calcifications.

Colour:

In general *parpam* are always white in colour but *Tankaparpam* (calx of Gold) is light yellow in colour

Character and tests for *parpam*:

- The final product should not have any glitter or shine.
- If a small quantity is pinched and rubbed between the thumb and index finger, the particles should be so fine as to enter and reside in the furrows and folds.
- If a pinch of *parpam* is gently put on the surface of water kept in a container, the material should not sink, but it should float.
- If the *parpam* is put into a crucible and heated, it should not be reverted into its metallic state.

Preservation and storage:

Parpam should be stored in a clean dry and air tight glass containers.

Shelf life:

Parpam keep their potency for 100 years.

Note:

- The colour effect and fineness of the *parpam* will be enhanced according to the degree of grinding and so it should be ground very finely.
- If the discs are not well dried, the *parpam* will not attain the specific colour specific for the particular *parpam*.
- When the discs (Dried *villai*) are arranged in the pans they should not be heaped up and should not be arranged in more than one layer. Only then the heat will react on the material properly. The pans should not be disproportionately big when compared to the quantity of the drugs and they should not be very deep also.
- The kiln is constructed by making circular excavations of suitable dimensions in places with optimum aeration and the sides are lined with bricks. Kilns should not be constructed in places where strong winds blow.
- Half of the numbers of cow dung cakes are spread at the bottom of the kiln and the calcination earthen vessels are placed over this at the centre. The remaining cow dung cakes are arranged over this and are to be ignited all around.
- Usually cow dung cakes are used as fuel in kilns. However in some specified instances some barks or goat dung and other materials are also recommended.

- In cow dung cakes there will be an appreciable admixture of sand or mud.Depending upon the degree to which there is such admixture the number and weight of the cow dung cakes are increased.One can determine this correctly only by adequate practical experience.
- Materials like sulphur and yellow orpiment which do not withstand strong heating are hidden in specified ashed when being calcined. In such cases the ash is spread in the pan, the discs placed over and then covered with more ash, after which the other pan is inverted over and scaled along the seam.
- The calcination capsules and the contents there in should be taken only when the kiln has cooled down by itself.

4. MATERIALS AND METHODS

PREPARATION OF THE DRUG:

GANDHAGA PARPAM has been selected from classical siddha literature Agasthiyar Vaithiya Vallathy – 600

Ingredients of the test drug are *Gandhagam, Sittoduvai*.

COLLECTION OF THE DRUG

The raw drugs *Gandhagam* were purchased from authorized drug store in Nagercoil at Kanyakumari district.

The herbal drug *Sittoduvai* were collected from Salem District.

IDENTIFICATION AND AUTHENTICATION OF DRUGS :

The raw materials were identified and authenticated by the experts of PG Gunapadam Dept, Government Siddha Medical College, Tirunelveli.

The identified raw materials were conserved in the laboratory of PG Gunapadam, Government Siddha Medical College, Tirunelveli.

INGREDIENTS

1) *Gandhagam (Sulphur) – S*
2) *Sittoduvai Juice (Odukkan) – Cleistanthus collinus (Linn)*

PURIFICATION OF INGREDIENTS

1) Gandhagam – (Sulphur):

Sulphur is placed in an iron spoon. A small quantity of cow's butter is added and the spoon is heated till the butter melts; this mixture is immersed in inclined position in cow's milk. This procedure is repeated for 30 time to get purified sulphur. Each time, fresh milk is to be used.

2. Sittoduvai (Odukkan):

Wash with clean water, leaves and tender stems are crushed and juice is extracted.

PREPARATION PROCESS

GANTHAGA PARPAM

Ganthagam is placed in stone morter and rubbed with sittoduvai juice for about 6 hours and made into cake. Next grind collinus leaves made into paste Ganthagam is placed within the oduvan paste and dried, then it is sealed with 7 clay clothes and dried into sunlight.

This is placed under the coarse sand and subjected into incineration process by using 96 cow dung cakes. After cooling ganthagam is taken again rubbed with *Odukkan* juice made into cake and dried. Then the leaves are made into paste then ganthagam is placed within the paste and dried. Then it is sealed with seven clay clothes and dried into sunlight.

This is again kept under coarse sand and subjected into incineration process by using 96 cowdung cakes. After cooling ganthagam is taken this process is repeated for about 3 times, obtained ganthagam cake is placed in a stone mortor and make into fine powder and store in a glass container.

Dosage :

488 mg (1 pannavedai)

Adjuvants:

Cow's ghee (or) Cow's milk

Shelf Life:

100 years

Indications

Putru (Cancer), *Kiranthi* (Syphilis), Leprosy, *Kandamaalai*, *Karappan*, *Soolai, Ooral, Pilavai.*

INGREDIENTS OF GANDHAGA PARPAM

GANDHAGAM

PURIFIED GANDHAGAM

INGREDIENTS OF GANDHAGA PARPAM

ODUKKAN

ODUKKAN JUICE

PROCESS OF GANDHAGA PARPAM

PREPARED DRUG

KAVASAMIDUTHAL

GANDHAGA PARPAM PREPARATION

PUDAMIDUTHAL

GANDHAGA PARPAM

4.2. STANDARDIZATION OF THE DRUG

4.2.1.PHYSICAL STANDARDIZATION AS PER *SIDDHA* CLASSICAL LITERATURE:

Standardization of drug means confirmation of it's quality and purity and detection of the nature of adulterant of various parameters like morphological, microscopic, physical, chemical and biological observations.

1. Colour:

The finished form of *Parpam* is white colour (Except Thanga parpam – It is yellow in colour)

2. Odour:

The finished form of *Parpam* is odourless ie, without any odour related to it's ingredients.

3. Taste:

A small amount of *Parpam* was kept in the tip of the tongue, which is tasteless. Properly prepared *Parpam* should be completely tasteless. If any taste present in *Parpam,* it indicates the preparation was not well prepared. It needs another *pudam* (incineration) process.

4. Finger Print Test:

Well prepared *Parpam* should be very fine. A pinch of *Parpam* was taken and rubbed in between the thumb and index finger. It entered into the depressions and furrows of the fingers.It confirmes the fineness of *Parpam.*

5. Floating on Water:

A pinch of *Parpam* was sprinkled over the water in a glass container. The *Parpam* particles did not sink but floated on the water surface. It indicates the lightness of *Parpam*.

6. Lustre:

If any glowing particles seen in the *Parpam* , it shows that the drug is not prepared properly and possess unchanged substances like metals and other toxic substances. So, there should be no glowing particles present in the properly prepared *Parpam*. The *Parpam* was taken in a Petri dish and observed for any lustre in daylight via magnifying glass.

4.2.2. STANDARDIZATION OF TEST DRUG BY USING MODERN TECHNIQUES:

Standardization of drug helps to authenticate and determine its quality and efficiency. Thus, the process involves qualitative and quantitative analysis by means of physico – chemical properties and instrumental analysis.

The physico – chemical analysis of *GANDHAGA PARPAM* has been done in iit Lab-chennai

The chemical finger prints are engaged by using modern analytical technique Fourier Transform Infra –Red Spectroscopy (FTIR) and Powder X-ray diffraction methods.

The particle size and qualitative analysis of chemical elements of *GANDHAGA PARPAM* are also assessed by Scanning Electron Microscope (SEM) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

4.2.3. PHYSICO CHEMICAL STANDARDIZATION

1. LOSS ON DRYING (INDIAN PHARMOCOEPIA, 1996)

Loss on drying is the loss in percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition. A glass stopper, shallow weighing bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle covered and weighed. The sample was distributed evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was removed from the chamber and allowed to cool at room temperature in a desiccators before weighing. The normal range is between 6 – 8 %.

2. TOTAL ASH:

Two grams of ground air dried powder of *GANDHAGA PARPAM* was accurately weighed in a previously ignited and tared silica crucible. The drug was gradually ignited by raising the temperature to 450^{o}C until it was white. The sample was cooled in a desiccators and weighed. The percentage of total ash was calculated with reference to air-dried drug.

a)Acid insoluble ash

The ash was boiled with 25ml of 2M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited cooled in a desiccators, and weighed. The percentage of acid insoluble ash calculated with reference to the air-dried drug.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol.

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o} to constant weight. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

MICROBIAL LIMIT TEST OF *GANDHAGA PARPAM*

Evaluation of Total Aerobic Bacterial Count

1.1. Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

1.2. Serial dilution of Sample

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as 10^{-1} dilution. From this dilution, 1ml was taken from that mixture is added to 9 ml, and designated as 10^{-2} dilution. The same procedure was repeated up to 10^{-4}.

1.3. Isolation of Total Viable Aerobic Microbial Count

1.3.1. Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45^{o}C, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of 10^{-1} and 10^{-2}. After inoculations, all the plates were incubated at 37^{o}C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected

1.3.1.1. Composition of Nutrient Agar Media

Peptone	: 5.0 gm
Sodium chloride	: 5.0 gm
Beef extract	: 1.5 gm
Yeast extract	: 1.5 gm

Agar	: 15.0 gm
Distilled water	: 1000 ml
pH (at 25°C)	: 7.4±0.2

1.3.2. Isolation of Fungi

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

1.3.2.1. Composition of SDA

Dextrose	: 40 gm
Peptone	: 10 gm
Agar	: 15 gm
Distilled water	: 1000 ml

1.4. Evaluation of Antimicrobial Activity of Drug

Antimicrobial activity was performed by agar well diffusion method on agar.

1.4.1 Preparation of drug extracts solutions for the experiment

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

1.4.2. Procedure for the Agar Well Diffusion Test

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

1.4.3. Bacterial Inoculums Preparation

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

1.4.4. Agar well-diffusion method

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

1.4.4.1. Composition of Muller Hinton Agar Media

Beef Extract	: 02.00 gm
Acid Hydrolysate of Casein	: 17.50 gm
Starch	: 01.50 gm
Agar	: 17.00 gm

1.5. Evaluation of Specified Microorganisms

1.5.1. Isolation & Identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.1.2. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

1.5.1.3. Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a water-bath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas. Then,

the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

1.5.1.4. Alternative test

It was done by a loop full of enriched culture in the primary test was streaked on a sterile Mac-Conkey agar medium. Then, the plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the pink or brick red color colonies were examined and transfer them individually into the surface of Eosin Methylene Blue agar medium (EMB), on Petri dishes. Inoculated plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the colonies on medium were checked for their color appearance like green metallic sheen under reflected light. The colonies were subjected to confirmation by further suitable cultural and biochemical tests.

1.5.1.5. Components of Eosin Methylene Blue Agar Media

Pancreatic digest of gelatin	: 10.0 g
Dibasic potassium phosphate	: 2.0 g
Lactose	: 10.0 g
Eosin Y	: 400 mg
Methylene blue	: 65 mg
Agar	: 15.0 g
Distilled water	: 1000 ml

1.5.2. Isolation & Identification of *Salmonella* sp.

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.2.1. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was subcultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After

incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

1.5.2.2. Secondary test

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

1.5.2.3. Composition of *Salmonella Shigella* Agar Media

Beef Extract	: 5.0 gm
Enzymatic Digest of Casein	: 2.5 g
Enzymatic Digest of Animal Tissue	: 2.5 gm
Lactose	: 10 gm
Bile salts	: 8.5 gm
Sodium Citrate	: 8.5 gm
Ferric Citrate	: 1.0 gm
Brilliant Green	: 0.00033 gm
Neutral Red	: 0.025
Agar	: 13.5 gm
Distilled water	: 1000 ml

1.5.3. Isolation and Identification of *Pseudomonas aeruginosa*

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

1.5.3.1. Composition of Cetrimide Agar Media

Pancreatic digest of gelatin	: 20.0 g
Magnesium chloride	: 1.4 g
Potassium sulphate	: 10.0 g
Cetrimide	: 0.3 g
Agar	: 13.6 g
Glycerin	: 10.0 g
Distilled Water t	: 1000 ml

1.5.4. Isolation and Identification of *Staphylococcus aureus*

From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37º C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

1.5.4.1. Composition of Mannitol Salt Agar Media

Pancreatic digest of gelatin	: 5.0 g
Peptic digest of animal tissue	: 5.0 g
Beef extract	: 1.0 g
D-Mannitol	: 10.0 g
Sodium chloride	: 75.0 g
Agar	: 15.0 g
Phenol red	: 25 mg
Distilled Water	: 1000 ml

4.2.4 BIO-CHEMICAL ANALYSIS

PROCEDURE:

5gms of the drug was wighted accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boilded well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is make up to 100ml with distilled water. This fluid is taken for analysis.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.5. PHYTOCHEMICAL ANALYSIS OF *GANDHAGA PARPAM*

Analysis of the siddha preparation *GANDHAGA PARPAM*

The siddha preparation *GANDHAGA PARPAM* was prepared and used for phytochemical analysis.

Preliminary test, on the siddha preparation *GANDHAGA PARPAM* was carried out for the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenolic compounds, proteins and free amino acids, flavanoids, lignin, fixed oils and fats. The methods adopted for the estimation are as follows:

1.Test for Alkaloids (Evans, 1997)

A small segment of the siddha preparation *GANDHAGA PARPAM* was mixed separately with a few drops of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various alkaloidal reagents as follows:

a) ***Mayer's test*** (Evans, 1997):

To a few ml of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates that the test as positive.

b) ***Hager's test*** (Wagner *et al*., 1996):

To a few ml of filtrate, one to 2ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive.

c) ***Dragendorff's test*** (Waldi, 1965):

To a few ml of filtrate, one to 2ml of Dragendorff's reagent is added. A prominent yellow precipitate indicates the test as positive.

2.Test for Carbohydrates (Ramakrishnan *et al*., 1994)

A small quantity of siddha preparation *GANDHAGA PARPAM* was dissolved separately in 5ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of 2 layers shows the presence of carbohydrates.

3.Test for Glycosides

The siddha preparation *GANDHAGA PARPAM* was hydrolyzed with hydrochloric acid for few h on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

(a) ***Legal's Test***:

To the hydrolysate, one ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides and aglycones.

(b) ***Borntrager's Test***:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink color, shows the presence of glycosides (Evans, 1997).

4.Test for Phytosterols (Finar, 1986)

(a) ***Liebermann Burchard Test*:**

Small amount of the siddha preparation *GANDHAGA PARPAM* was dissolved with 3ml of acetic anhydride, a few drops of glacial acetic acid and followed by the addition of few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterols.

(b) ***Salkowski Test*:**

Small quantities of the siddha preparation *GANDHAGA PARPAM* were dissolved in chloroform separately. This chloroform solution was added with few drops of concentrated sulphuric acid. The appearance of bluish green color shows the presence of phytosterols.

5.Test for Saponins (Kokate, 1999)

Frothing Test:

The siddha preparation *GANDHAGA PARPAM* was diluted separately with 20ml of distilled water and it was agitated on a graduated cylinder for 15min. Absence of the foam formation shows the devoid of saponins

6.Test for Phenolic Compounds and Tannins (Mace, 1963)

Small quantities of siddha preparation *GANDHAGA PARPAM* was dissolved separately in water and tested for the presence of phenolic compound and tannins. In the process of testing and treating, the following observations were noted:

a) Dilute ferric chloride solution (5%) gives a dark green color. 38

b) 10% aqueous potassium dichromate solution gives yellowish brown precipitate.

c) 10% lead acetate solution gives a white precipitate.

7. Test for Proteins and Free Amino Acids (Fisher, 1968; Ruthmann, 1970)

Small quantities of various siddha preparation *GANDHAGA PARPAM* was dissolved in few ml of water and the following reaction were carried out

(a) *Millon's Test* :

To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins (Rasch and Swift, 1960).

(b) *Ninhydrin Test*:

To 2ml of filtrate 2 drops of ninhydrin solution was added. A characteristic purple color indicates the presence of amino acids (Yasma and Ichikawa, 1953).

(c) *Biuret Test*:

An aliquot of 2ml of filtrate was treated with a drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, Pink color in the ethanol layer indicates the presence of protein (Gahan, 1984).

8.Test for Flavanoids

(a) ***Shinoda's Test*:**

Small quantity of siddha preparation *GANDHAGA PARPAM* was treated with alcohol to that a piece of magnesium was added followed by an addition of concentrated hydrochloric acid drop wise and heated. Appearance of magenta color shows the presence of flavanoids (Harborne, 1984).

(b) ***Florescence Test*:**

Small quantity of *GANDHAGA PARPAM* was dissolved separately in alcohol and a drop of that extract was placed on Whatman filter paper and observed under UV light. Florescence indicates the presence of flavanoids.

9. Tests for Lignin

Small quantities of *GANDHAGA PARPAM* was dissolved separately in few ml of alcoholic solution of hydrochloric acid and phloroglucinol gives red color, which shows lignin is present.

10. Tests for Fixed oils and Fats

(a) ***Spot Test*:**

A small quantity of siddha preparation *GANDHAGA PARPAM* was placed between 2 filter papers. Oil stains produced with any extract shows the presence of fats and fixed oils in the *GANDHAGA PARPAM* (Harborne, 1984).

(b) ***Saponification Test*:**

A small quantity of siddha preparation *GANDHAGA PARPAM* was treated with few drops of 0.5N alcoholic potassium hydroxide along with 2 to 3 drops of phenolphthalein. Later the mixture is refluxed for about 2h. Soap formation indicates the presence of fats and fixed oils in the *GANDHAGA PARPAM*.

4.2.6. INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)

Fig. No. 1 Scanning Electron Microscope (SEM)

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope

Introduction:

The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter

Principle:

The beam is then rastered over the specimen in synchronism with the beam of a cathode ray tube display screen. The eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which

is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

SEM MECHANISM

Procedure:

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen issufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons Colliding with elemental components of the specimen and releasing secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the

peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

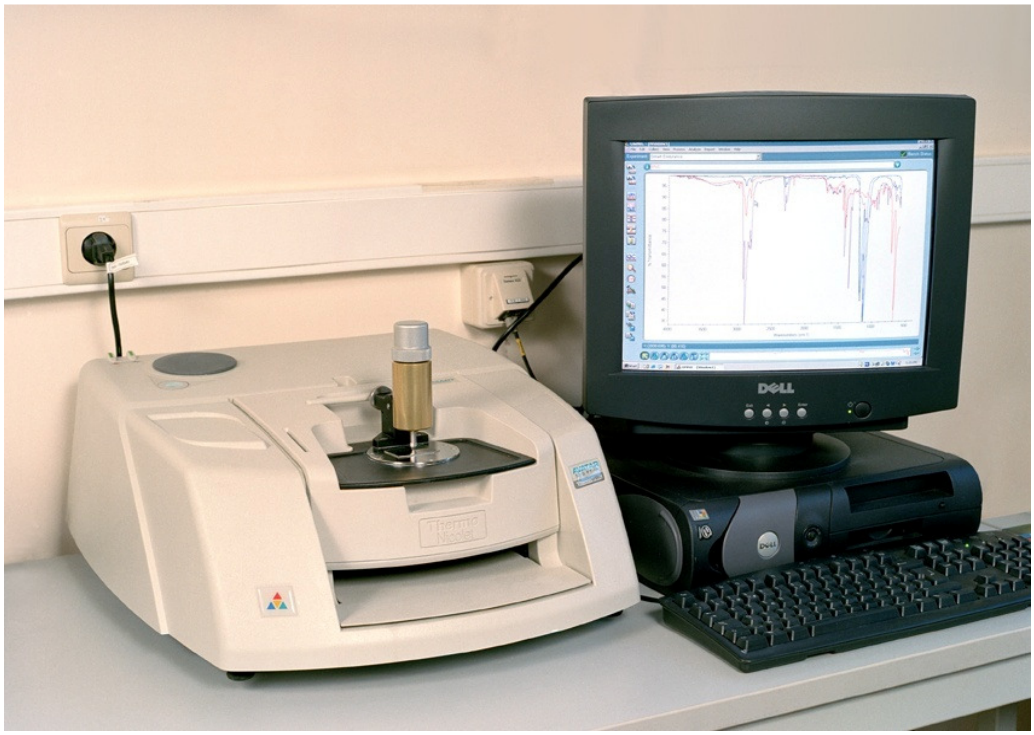
In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields.The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *GANDHAGA PARPAM*. SEM results of *GANDHAGA PARPAM* were represented in results section.

Fig. No. 2 FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)



Introduction:

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure .The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle:

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source : Nernst Glower

Beam splitter : It is made up of a transparent material. Thin films of Silicondepositedon Potassium bromide (KBr) Bromide (KBr) Detectors: DeutratedTriGlycine Sulphate (DTGS).

Scan Range : MIR 450to 4000 cm-1

Resolution : 4.0 cm-1

Sample required : 50mg, solid or liquid

Sampling Techniques: There are a variety of techniques for sample preparation physical form of the sample to be analyzed.

Solid : KBr or Nujol mull method.

Liquid : Csl / TIBr Cells

Gas : Gas cells

Measurements Techniques:

The procedure for recording the %T or %A is as follows:

1) Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required % T or % A at various frequencies.
2) Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
3) Small amount of samples are sufficient
4) High resolution is obtained.

Procedure:

Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

1) Then, 300 Al of 20 mM buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the This buffer.
2) The washings took 24 h, which is the time of contact of the protein with the 2H20medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuterated triglycinesulfate detector.
3) At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40^oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5^oC steps from 20 to 95^oC.
4) Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm_ 1

KBr Method

1) The sample is grounded using an agate mortar and pestle to give a very fine powder.
2) The finely powder sample is then mixed with about 100mg dried KBr salt.
3) The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method:

1) The sample is ground using an agate mortar and pestle to give a very fine powder.
2) A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
3) The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

1) Viscous liquids can be smeared in the cell and directly measured.
2) For dilute solutions, liquid cells and variable path length cells are employed.

Applications:

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

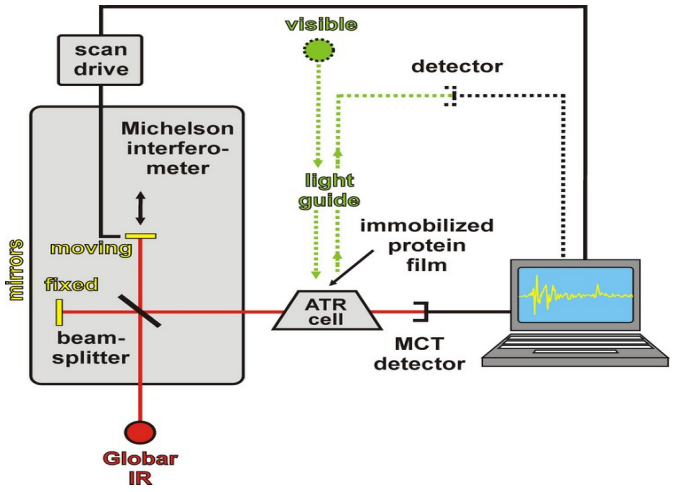


Fig. No. 3 Mechanism of FTIR analyzer

Analytical Capabilities:

1) Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.
2) Especially capable of identifying the chemical bonds of organic materials
3) Detects and identifies organic contaminants.
4) Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
5) Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

Fig. No. 4 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY(ICP-OES):

Introduction:

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

Mechanism:

The ICP-OES is composed of two parts: ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma.

When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "workcoil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that

creates a brief discharge are through the argon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. Stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved.

Within the optical chamber(s), after the light is separated into its different wavelengths (colours), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved, or, in more modern units, the separated colours fall upon an array of semiconductor photo detectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyse for every element to which the unit is sensitive all at once. Thus, samples can be analysed very quickly. The intensity of each line is then compared to previously measured intensities ofknown concentrations of the elements and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix.

Applications :

ICP-OES is used in the determination of metals, arsenic present in Traditional medicines, and trace elements bound to proteins. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.

The author used it for elemental identification and quantitative compositional information of the *GANDHAGA PARPAM* **.**

X-RAY POWDER DIFFRACTION
(XRD) INSTRUMENTATION

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder and an X-ray detector.

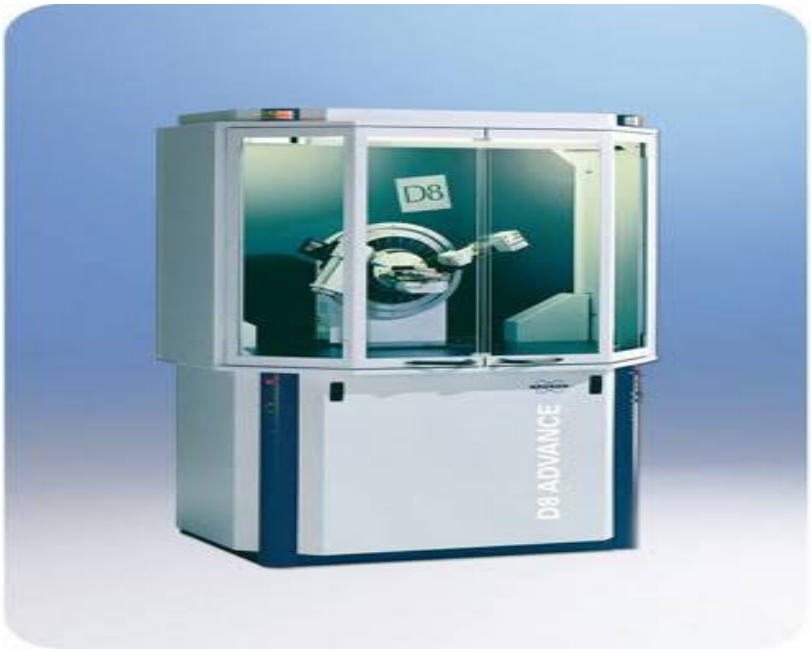


Fig.No. 5 Bruker's X-ray Diffraction D8-Discover instrument.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons towards a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_α and K_β. K_α consists in part of $K_{\alpha1}$ and $K_{\alpha2}$. $K_{\alpha1}$ has a slightly shorter wavelength and twice the intensity of $K_{\alpha2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha1}$ and $K_{\alpha2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_α radiation = $1.5148A^{0.}$ These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and

processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from -5^0 to 70^0, angles that are present in the X-ray scan.

Applications:

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

1) Characterization of crystalline materials
2) Identification of the fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically.
3) Determination of unit cell dimensions.
4) Measurement of sample purity.

With specialized techniques, XRD can be used to:

1) Determine crystal structures using Rietveld refinement
2) Determine of modal amounts of minerals (quantitative analysis)
3) Make textural measurements such as the orientation of grains in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

1) Powerful and rapid (<20 min) technique for identification of an unknown minerals.
2) In most cases, it provides an unambiguous mineral determination.
3) Minimal sample preparation is required.
4) XRD units are widely available.
5) Data interpretation is relatively straight forward.

Limitations:

1) Homogenous and single phase material is best for identification of an unknown
2) Must have access to a standard reference file of inorganic compounds (d-spacings, *hkls)*
3) Requires tenths of a gram of material which must be ground into a powder.
4) Foe mixed materials, detection limit is -2% of sample.
5) For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.
6) Peak overlay may occur and worsens for high angle 'reflections'.

User's Guide-Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding and a sample holder.

1. Obtain a few tenths of a gram (or more) of the material, as pure as possible.
2. Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
    i. Powder less than -10 μm (or 200-mesh) in size is preferred.
2. Place into a sample holder or onto the sample surface.
    i. Packing of the fine powder into a sample holder. Smear uniformly onto a glass slide, assuring a flat upper surface.
3. Pack into a sample container
4. Sprinkle on double sticky tape
    i. Typically the substance is amorphous to avoid interference
5. Care must be taken to create a flat upper surface and to achieve a random distribution of lattice orientations unless creating an oriented smear.
6. For unit cell determinations, a small amount of a standard with known peak positions (that do not interfere with the sample) can be added and used to correct peak positions.

Data Collection, Results and Presentation:

Data collection:

The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections ($K_{\alpha 1}$ and $K_{\alpha 2}$), at small values of 2θ the peak locations overlap with $K_{\alpha 2}$ appearing as a hump on the side of $K_{\alpha 1}$. Greater separation occurs at higher values of θ. Typically these combined peaks are treated as one. The 2λ position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Data reduction:

Results are commonly presented as peak positions at 2θ and X-ray counts (intensity) in the form of a table or an *x-y* plot (shown above). Intensity (*I*) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (*relative intensity =I/I_1 x 100).*

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of λ. Once all d-spacings have been determined, automated search/match routines compare the *ds* of the unknown to those of known materials. Because each mineral has a unique set of d-spacings, matching these d-spacings provides an identification of the unknown sample. A systematic procedure is used by ordering the d-spacings in terms of their intensity beginning with the most intense peak. Files of d-spacings for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction File (PDF). Many other sites contain d-spacings of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

The author used it for elemental identification and quantitative compositional information of the *GANDHAGA PARPAM.*

4.3. TOXICOLOGICAL STUDIES

4.3.1. ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *GANDHAGA PARPAM*

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance ***GANDHAGA PARPAM*** , when administered orally to Female Wister Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

(a) OECD Guidelines No. 423,

Study Design and Controls:

1. Female Wister Rats in controlled age and body weight were selected.
2. ***GANDHAGA PARPAM*** was administered at **5 mg/kg, 50 mg/kg, 300 mg/kg,1000 mg/kg, 2000 mg/kg,** body weight as (Water) as suspension along with blank.
3. The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from Central Animal House, Arulmigu Kalasalingam college of pharmacy, krishnankoil. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

2. DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

Water

The water was offered ad libitum in bottles.

3. ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wister Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table – 1 Numbering and Identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

GROUP	DOSE
Group-I	5 mg/kg
Group-II	50 mg/kg
Group-III	300 mg/kg
Group-IV	1000 mg/kg
Group-V	2000 mg/kg

The test item was administered as single dose. After single dose administration period, all animals were observed for day 14.

Dose Preparation

GANDHAGA PARPAM was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each Female Wister rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

4.3.2. SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY PROFILE OF *GANDHAGA PARPAM*

Objective

The objective of this '**Sub-Acute Toxicity Study of *GANDHAGA PARPAM* on Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

Test Guideline Followed

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

Test Item Detail

GANDHAGA PARPAM

Test System Detail

The study was conducted on 5 male 5 female Wister rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at Central Animal House, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 male and 5 male animals per group.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table – 2 Numbering and Identification

Case No	**Group No**	**Animal Marking**
1	CONTROL	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female
2	Low dose of *GANDHAGA PARPAM* 300mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
3	Middle dose of *GANDHAGA PARPAM* 600mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
4	High dose of *GANDHAGA PARPAM* 900 mg/kg	H,B,T,HB,NM (Male) H,B,T,HB ,NM (Female)

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Case No	**Group No**	**Animal Marking**	**Sex**
1	CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2	Low dose of *GANDHAGA PARPAM* 300mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3	Middle dose of *GANDHAGA PARPAM* 600mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4	High dose of *GANDHAGA PARPAM* 900 mg/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

Husbandry

Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 5 rats of the same sex and treatment group.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22±3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

Feed & feeding schedule

Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

Test Group	**Dose to Animals (mg/kg body –weight / day**	**Number of Animals**
Group – I	CONTROL	10 (5 Male and 5 Female)
Group – II	Low dose of *GP* 300mg/kg	10 (5 Male and 5 Female)
Group – III	Middle dose of *GP* 600mg/kg	10 (5 Male and 5 Female)
Group - IV	High dose of *GP* 900 mg/kg	10 (5 Male and 5 Female)

The test item was administered as single dose. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

GANDHAGA PARPAM was added in distilled water and completely dissolved to for oral for administration. The dose was prepared of a required concentration before dosing by dissolving ***GANDHAGA PARPAM*** in distilled water.

It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 10 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN ect……...

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin (g %)
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles (x103/cmm)
RBC	:	Red Blood Corpuscles (x106/cmm)
		Blood Platelet count (x103/cmm)

Differential WBC count:

N	:	Neutrophils (%)
L	:	Lymphocytes (%)
M	:	Monocytes (%)
E	:	Eosinophils (%)
RDW	:	Red Cell Distribution Width.
MPV	:	Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT	:	Alanine amino transferase (U/L)
AST/SGOT	:	Aspartate amino transferase (U/L)
ALP	:	Alkaline serum phosphatase (U/L)
CHL	:	Cholesterol (mg/dL)
HDL	:	High density lipoprotein
TG	:	Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

PHARMACOLOGICAL ANALYSIS

ANTI CANCEROUS ACTIVITY OF *GANDHAGA PARPAM ON* DALTON'S LYMPHOMA ASCITES IN MICE

Induction of cancer using DLA cells

Dalton's Lymphoma ascites (DLA) cell was supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained in vivo in Swiss albino mice by intraperitoneal transplantation. While transforming the tumor cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilution were made so that total cell should be 1 x 10^6, this dilution was given intraperitonealy. Let the tumor grow in the mice for minimum seven days before starting treatments.

Treatment Protocol

Swiss Albino mice were divided in to five group of six each. All the animals in four groups were injected with DLA cells (1 x 10^6 cells per mouse) intraperitonealy, and the remaining one group is normal control group.

Group 1 served as the normal control.

Group 2 served as the tumor control. Group 1 and 2 receives normal diet and Water.

Group 3 served as the positive control, was treated with injection fluorouracil at 20 mg/kg body weight, Intra peritoneally.

Group 4 **Served** as a treatment control group and was Administered ***GP*** at a dose of 200mg/kg through orally. (LD_{50} OECD Guidelines)

Group 5 Served as a treatment control group and was Administered ***GP*** at a dose of 200mg/kg through orally. (LD_{50} OECD Guidelines)

Treatment

In this study, drug treatment was given after the 24 hrs of inoculation, once daily for 14 days. On day 14, after the last dose, all mice from each group were sacrificed; the blood was withdrawn from each mouse by retro orbital plexus method and the following parameters were checked.

1. Hematological parameters
    1) WBC count
    2) RBC count

3) Hb content
4) Platelet count
5) Packed cell volume

2. Serum enzyme and lipid profile
   3. Total Cholesterol (TC)
   4. Triglycerides (TG)
   5. Aspartate amino Transferase (AST)
   6. Alanine amino Transferase (ALT)
   7. Alkaline Phosphatase (ALP)

3. Derived parameter
   1. Body weight
   2. Life span (%)
   3. Cancer Cell Count

EVALUATION OF CLINICAL PARAMETERS

Cancer cell count

The fluid (0.1ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of ice cold Normal saline or sterile Phosphate Buffer Solution and 0.1 ml of tryphan blue (0.1 mg/ml) and total numbers of the living cells were counted using heamocytometer.

$$\text{Cell count} = \frac{\text{No of cells Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

6) Hematological parameters

i) WBC count
ii) RBC count
iii) Platelet count
iv) Hemoglobin
v) Packed Cell Volume

i) WBC count

The total WBC count was found to be increased in cancer control, when compared with normal and treated tumor-bearing mice. The total WBC count were found to decrease significantly in animals treated with extract when compared with cancer control, indicating that the antitumor nature of the extract.

ii) RBC and Hb

RBC and Hb content decreases with tumor bearing mice when compared with Normal control mice.

iii) Platelets

In Hodgkin lymphoma, increased in platelet count often reported in laboratory finding. Hence, I investigated this parameter in the study.

iv) Packed cell volume

In any case of anemia the packed cell volume is decreases.

SERUM ENZYME AND LIPID PROFILE

The serum was analyzed for the following parameters

1. Aspartate amino Transferase (AST)
2. Alanine amino Transferase (ALT)
3. Alkaline Phosphatase (ALP)
4. Total Cholesterol (TC)
5. Triglyceride (TG)

1. TOTAL CHOLESTEROL AND TRIGLYCERIDE (lipid profile)

Abnormal blood lipid profile has been associated with cancer. In Hodgkin lymphoma, high cholesterol level and low triglyceride level has been reported. Hence I investigated this parameter in the study.

2. LIVER ENZYMES (AST, ALT, ALP).

Abnormal liver function seen in patient with Hodgkin lymphoma, that these liver enzyme levels markedly increase in tumor bearing mice. ALP is an enzyme mainly derived from the liver, bones and in lesser amount from intestines, placenta, kidneys and leukocytes. An increase in ALP levels in the serum is frequently associated with the variety of disease. ALP comprises a group of enzyme that catalyzes the phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate.

Markedly elevated serum ALP, hyperalakline-phosphatasemia, is seen predominantly with more specific disorders; including malignant biliary cirrhosis, hepatic lymphoma and sarcoidosis. Hence, I investigated this parameter in this study.

DERIVED PARAMETERS

1. Body weight:

All the mice were weighed, from the beginning to 15th day of the study. Average increase in body weight on the 15th day was determined.

2. Percentage increase in life span (ILS)

- ❖ All biochemical investigations were done by using COBAS MIRA PLUS-S Auto analyzer from Roche Switzerland.
- ❖ Hematological test are carried out in COBAS MICROS OT 18 from Roche.
- ❖ Newly added Hi-Tech instruments MAX MAT used for an auto analyzer for all biochemistry investigations in blood sample.

Induction of Cancer Using DLA Cell

Dalton's Lymphoma Ascites (DLA) cells were supplied by Amala cancer research center, Trissur, Kerala, India. The cells were maintained in vivo in Swiss albino mice by intraperitoneally transplantation. DLA cells aspirated from the peritoneal cavity of mice were washed with saline and given intraperitoneally to develop carcinoma.

Effect of GP on Survival Time

Animals were divided into five groups of six animals each. Except the normal control group, the remaining groups were inoculated with DLA cells ($1x10^6$ cells/mouse)intraperitoneally on day '0' and treatment with GP started 24 hrs after inoculation, at a dose of 200mg/kg/day. *p.o*. The normal and tumor control group was treated with same volume of 0.9% sodium chloride solution. All the treatments were given for fourteen days. The increase in life span (ILS) of each group, consisting of 6 mice was noted.

The antitumor efficacy of GP was compared with that of 5-fluorouracil (Dabur pharmaceutical ltd. India; 5-FU, 20 mg/kg/day, *i.p,* for 14 days). The ILS of the treated groups was compared with that of the control group using the following calculation:

Increase in lifespan = [(T - C) / C] x 100

Where T = number of days the treated animal survived.

C = number of days control animals survived.

EVALUATION OF ANTIOXIDANT ACTIVITY OF *GANDHAGA PARPAM* THROUGH DPPH (2, 2-DIPHENYL 1-2 PICRYLHYDRAZYL) ASSAY

The antioxidant activity of *GANDHAGA PARPAM* was determined using the 2, 2-diphenyl-1 picrylhydrazyl (DPPH) free radical scavenging assay .100µl of *GANDHAGA PARPAM* extract was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control subsequently, at every 5 min interval, the absorption maximum of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates.

Free radical scavenging activity of *GANDHAGA PARPAM* calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs of Control} - \text{Abs of Test})}{\text{Abs of Control}} \times 100$$

ANTI-INFLAMMATORY ACTIVITYOF GANTHAGA PARPAM BY CARRAGEENAN INDUCED HIND PAW EDEMA METHOD

The anti-inflammatory activities of **GANTHAGA PARPAM** at 100 mg/kg& 200 mg/kg doses were evaluated using carrageenan-induced paw edema method. The inflammation was readily produced in the form of edema with the help of irritant such as carrageenan. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and when injected cause the release of prostaglandins by the way it produces inflammation and edema.

REQUIREMENTS:

Animal	:	Albino rat (180-200 g)
Drugs and chemicals	:	Carrageenan (1%w/v), Diclofenac sodium (standard), Carboxy methyl cellulose (1%w/v), Plethysmo meter.
Test compounds	:	**GANDHAGA PARPAM**

METHOD:

Anti-inflammatory activity was performed by the following procedure of Bhandri et al(1)The animals were divided into 4 groups, each groups having 6 animals. A freshly prepared suspension of carrageenan (1% w/v , 0.1 ml) was injected to the planter region of left hind paw of each rat. One group was kept as control and the animals of the other groups were pretreated with the **GANDHAGA PARPAM** test Compounds dissolved with 2 ml sterile water given through orally 30 min before the carrageenan treatment. The paw volumes of the test compounds, standard and control groups were measured at 60,240,360 minutes of carrageenan treatment with the help of plethysmometer. Mean increase in paw volume was measured and the percentage of inhibition was calculated.

% Anti-inflammatory activity = (Vc-Vt / Vc)* x *100

Where, ***Vt***-mean increase in paw volume in rats treated with test compounds,

Vc-mean increase in paw volume in control group of rats.

5. MICROBIOLOGICAL ANALYSIS

ANTI - MICROBIAL STUDIES

Aim

To study the Anti-microbial action of "*GANDHAGA PARPAM* " done by **"Agar well diffusion method"** – Kirby – bauyermethod.

Components of Muller Hinton agar medium

Beef extract	-	300gms/lit
Agar	-	17 gms/lit
Starch	-	1.5 gms/lit
Casein Hydrolysate	-	17.5 gms/lit
Distilled water	-	1000 ml
PH	-	7.6

Procedure:

The method of antibacterial activity study is UPS Diffusion Method. Antibiotic discs are prepared with known concentration of antibiotic are placed on agar plates that has been inoculated with the known pathogenic micro organism. The antibiotic diffuses through the agar producing an antibiotic concentration, gradient antimicrobial susceptibility is proportional to the diameter of the inhibitory zone around the disc. If the microorganism which grows up to the edge of the disc are resistant to the antimicrobial agent. The recommended medium in this method is Muller Hinton Agar, its PH should be between 7.2-7.6 and should be poured to uniform thickness of 4mm in the petri plate (25ml).

Methodology:

Muller Hinton Agar plates are prepared and Pseudomonas, Staphylococcus aureus, Escherichia coli, Candida Species is inoculated separately.

The prepared disc of *GANDHAGA PARPAM* are placed over the incubated plate using sterile forceps and incubated for 24 hours at 37°C. The plates after 24 hours incubation are observed for the zone of inhibition.

6. RESULTS AND DISCUSSION

6.1. STANDARDISATION OF *GANDHAGA PARPAM*

The test drug ***GANDHAGA PARPAM*** had been subjected to various studies to establish the works of Siddhar`s to be true. Literary collections, physico-chemical and elemental analysis, pharmacological study, toxicological study and antimicrobial study are done to prove the activity of ***GANDHAGA PARPAM*** as an anti-diarrhoeal, anti-spasmodic and anti-pyretic activities.

Table – 3 Physico Chemical Standardisation.

SL. NO.	PARA METER	RESULTS
1.	Organo leptic characters	
	1. Color	Grey
	2. Odour	Pleasant odour
	3. Sense of touch	Soft
	4. Taste	Bitter astringent
	5. Appearance	Powder
1.	Physico chemical standard	
	1. Loss on drying at 70°C	7.30 %
	2. Ash	
	a. Total Ash	8.25%
	b. Acid insoluble ash	0.95 %
	c. Water soluble	7.75 %
	3. Extractive value	
	a. Ethanol soluble extractive	8.10 %
	b. Water soluble extractive	9.30 %
	4. pH value (1% solution)	7.540

Interpretation:

The physical parameters like colour, odour touch, appearance revealed that *GANDHAGA PARPAM* is a Grey, Pleasant odour, having the PH 7.540 slightly alkaline Ph.

Determination of loss of drying normal:

The loss of drying test is designed to measure the amount of volatile matters in a sample when the sample is dried under specified conditions moisture is one of the major factors. Responsible for the deterioration of the drugs and formulations low moisture content is always desirable for higher stability of days.

The percentage of loss on drying was within acceptable range to thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

Total Ash:

Ash values are helpful in determining the quality and purity of crude drugs. in this trial drug *GANDHAGA PARPAM* (The minerals that present in the trial drug are calcium, chloride, sulphate). The salts, Ca+, Cl-, Sulphate are not harmful one. In this trial drug *GANDHAGA PARPAM*is used as a condensation from water extraction . So only water soluble trace elements present here in a very few trace levels. The total ash was 8.25%.

Acid insoluble Ash:

Acid insoluble ash values represents detecting the presence of silica and oxalate in a drugs. In my drug the silica and oxalate that is the acid insoluble ash is very low on 0.95 ± 0.011. So the drug has high quality.

Water soluble ash:

Water soluble ash also indicate the purity of the drug water soluble ash higher than acid insoluble ash represents good quality of the drug which is *GANDHAGA PARPAM* is 7.75 %. So water soluble ash is higher than acid insoluble ash.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol water soluble extractive *GANDHAGA PARPAM* is 9.30 %.

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o},to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug. Alcohol soluble extractive *GANDHAGA PARPAM* is 8.10 %.

d)Determination of pH

5 gms of *Ganthaga Parpam* was weighted accurately and placed in clear 100ml beaker. Then 50ml of distilled water was added to it and dissolved well. after 30 minutes it was then applied into pH meter at standard buffer solution of 4.0, 7.0 and 9.0. Repeat the test 4 times and average was recorded. The pH of *GANDHAGA PARPAM* is 7.540.

Microbial Limit Tests

Results of Microbial Contamination Test

S.No.	Test Particulars	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	Total Viable Aerobic Bacterial Count	4×10^2	1×10^5
2.	Total Viable Fungal Count	3.5×10^2	1×10^3

Results of Specific Pathogens Test

S.No.	Test for Specified Pathogens	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	*Salmonella* sp.	No growth	-
2.	*Staphylococcus aureus*	No growth	-
3.	*Escherichia coli*	No growth	-
4.	*Pseudomonas aeruginosa*	No growth	-

Interpretation:

The total bacterial count and the total fungal count of the drug were found to be within the WHO prescribed limits which indicate that the drug is free from microbial contamination. The other pathogens like Escherichia coli, Salmonella sps, Pseudomonas aeruginosa and Staphylococcus aureus were found to be completely absent in the drugs.

Disintegration:

The disintegration of test sample under the specifications not more than 15 minutes. In the present analysis the *GANDHAGA PARPAM* disintegration on only 10m 50 sec.

BIO-CHEMICAL ANALYSIS

Table – 4 Results of Preliminary test for basic and acidic radicals

S.NO	EXPERIMENT	INFERENCE
1.	Test for Calcium	Absent
2.	Test for Sulphate	**Present**
3.	Test for Chloride	**Present**
4.	Test for Carbonate	Absent
5.	Test for Starch	Absent
6.	Test for Ferric Iron	Absent
7.	Test for Ferrous Iron	Absent
8.	Test for Phosphate	Absent
9.	Test for Albumin	Absent
10.	Test for Tannic Acid	Absent
11.	Test for Unsaturated Compounds	Absent
12.	Test for Reducing Sugar	Absent
13.	Test for Amino Acid	Absent
14.	Test for Zinc	Absent

INTERPRETATION:

The biochemical analysis of *GANDHAGA PARPAM* contains the following chemical constituents, Sulphate, Chloride.

SULPHATE:

- It significantly improves the joint function. It helps in reduce the pain and inflammation.
- Sulphate's primary biological role in halting or reversing joint degeneration.
- Nutritionally essential element
- Functional in the form of sulphur containing amino acids.

CHLORIDE:

- Calcium- activated chloride channels (Ca-Cl) are thought to regulate neuronal excitability and recently chloride regulation in DRG (Dorsal root ganglion) neurons has attracted much attention in pain research.
- Chloride forms the chief anion of the extracellular fluid and exists along with sodium mostly.
- Regulates acid base balance.
- Formation of HCl in gastric juice
- Help to preserve normal neuromuscular irritability by maintaining a state of equilibrium, on account of their relative proportion in ECF and ICF.

PHYTOCHEMICAL STUDY OF *GANDHAGA PARPAM*

The *GANDHAGA PARPAM* was subjected to qualitative chemical investigation Details of the various tests performed for the presence of phyto constituents is shown in Table 5.

Table – 5 Phytochemical tests for *GANDHAGA PARPAM*

Tests	*GANDHAGA PARPAM*
Alkaloids	
Mayer's test	-ve
Dragendroff's test	-ve
Hager's test	+ve
Carbohydrates and glycosides	
Molisch test	+ve
Legal's test	-ve
Borntrager's test for anthraquinones	-ve
Phytosterols	
Liebermann-Burchard test	-ve
Salkowski test	-ve
Flavanoids	
Shinoda test Magnesium turnings and hydrochloric acid (Presence of red color)	-ve
Fluorescence test	-ve
Tannins	
Ferric chloride test	-ve
Potassium dichromate test	-ve
Lead acetate test	+ve
Proteins	
Millon's test	-ve
Biuret test	-ve
Ninhydrin test	-ve
Fixed oils and fats	
Spot test	-ve
Saponification test	-ve
Lignin	
Phloroglucinol test	-ve
Saponins	
Frothing test	-ve

(+ve) indicates the presence of phytochemical, (-ve) indicates the absence of phytochemical.

INTERPRETATION:

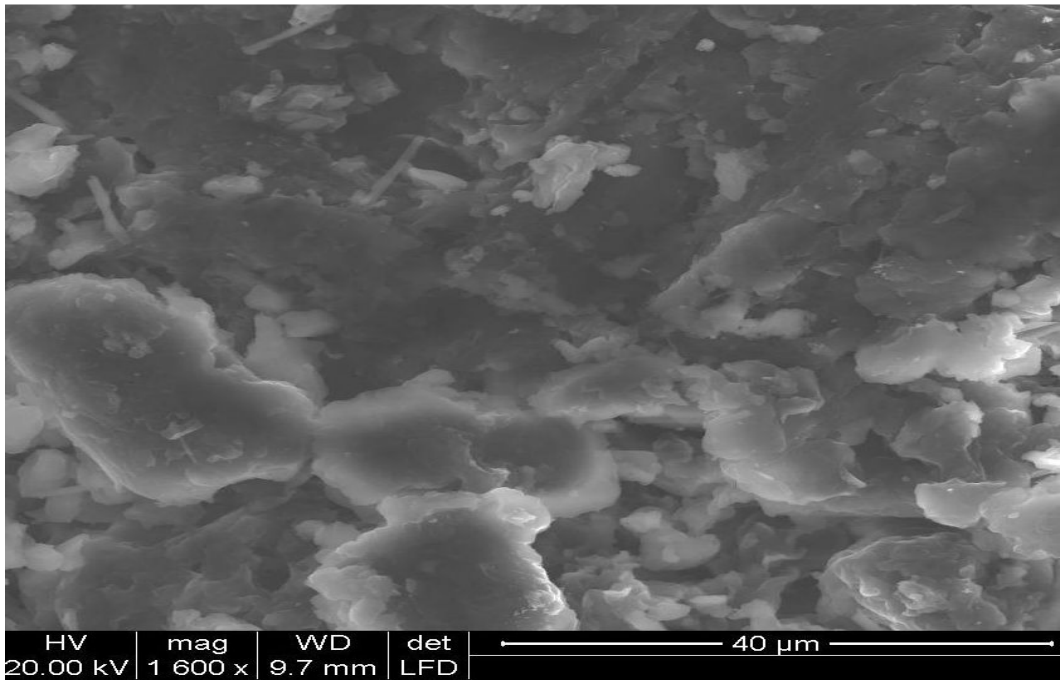
Alkaloids-decreased gastric acid secretion and inhibit the gastric motility

Tannins-Tannins react with tissue promote tissue proteins.

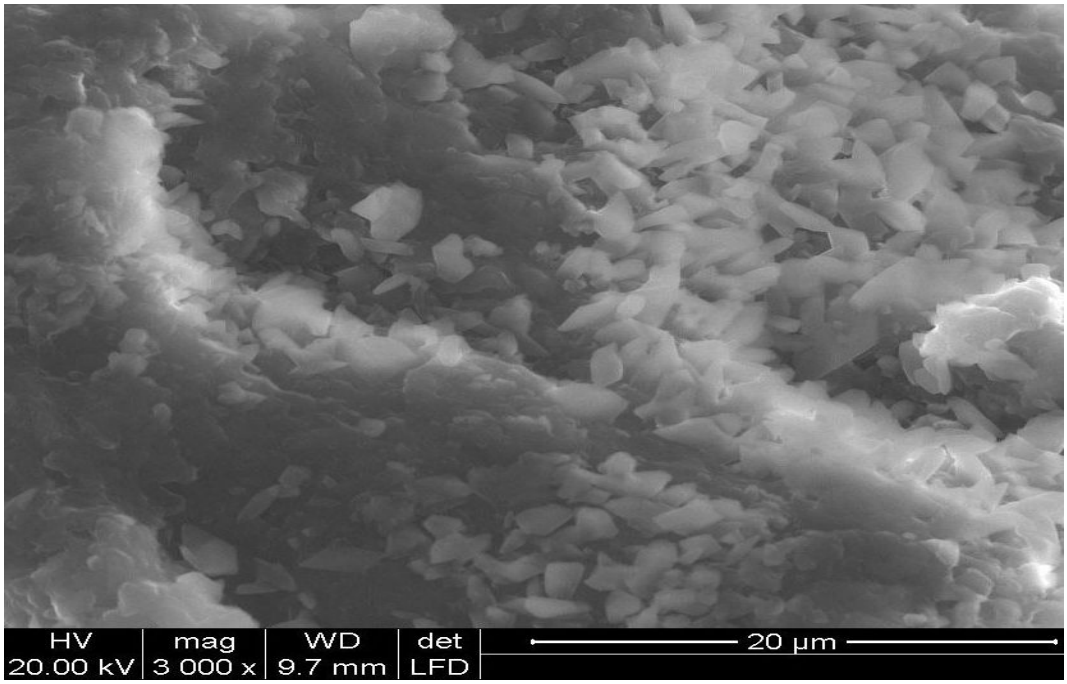
This study revealed the presence of active phytochemicals in *GANDHAGA PARPAM* such as alkaloids, carbohydrates, glycosides, tannins.

INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)



SEM -1600 Magnification



SEM -3000 Magnification

Figure - 6 Showing SEM Results of Trial Drug

(GANDHAGA PARPAM)

INTERPRETATION :

The morphology of the *GANDHAGA PARPAM*samples can be determined by Environmental SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination. The SEM photographs revealed that particles were spherical in shapes and sizes were in the range from 1µm to 5µm.Although the particle sizes of different batches showed similarity, it seems that these particles were aggregates of much smaller particles.

When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gave these particles a tendency to aggregate together to form larger particles. ***GANDHAGA PARPAM*** exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation. SEM analysis of the ***GANDHAGA PARPAM*** shows most of the particles present in the sample are nano size, average particle size is **40-20µm**.

FOURIER TRANSFORM-INFRARED SPECTROSCOPY(FTIR)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra that provide information about the functional group and molecular structure of a material IR relates with the sample and the bonds among atoms in the molecule stretch and bend, absorbing infrared energy and creating the infrared spectrum. It is of two kinds of bending and stretching.

FT-IR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs. The results of Table no: 6 and Fig no: 7 shows the presence of functional group and inorganic compounds of ***GANDHAGA PARPAM***

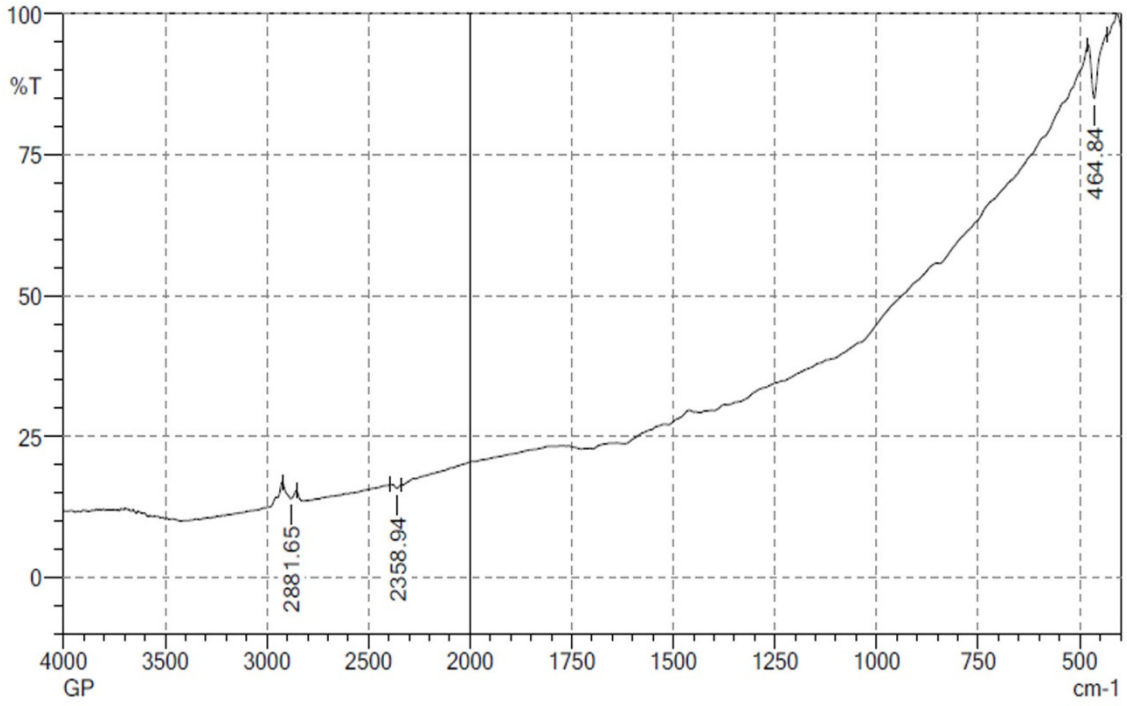


Figure -7 Showing FTIR Image of *GANDHAGA PARPAM*

Table – 6 Interpretation of FTIR Spectrum

S.No	Frequency	Bond	Functional Group
1.	2881.65	C-H Stretching	Alkane
2.	2358.94	O=C=O Stretching	Carbon-di-oxide
3.	464.84	C-X Stretching	Bromide, Iodide

INTERPRETATION:

1. FTIR instrumental analysis was done. The test drug was identified to have 3 peaks. They are the functional groups present in the trial drug *GANDHAGA PARPAM*
2. It confirms that *GANDHAGA PARPAM* constitutes Alkanes, Carbon-di-oxide, bromide, Iodide as functional groups.

ALKANES:

They protect against bacteria and fungal infections.

BROMIDE:

Bromides were widely used as sedative and antiepileptic. Bromide ion causes secondary anion potentiation of gamma – aminobutyric acid (GABA) channels in the CNS.

ICP-OES of *GANDHAGA PARPAM*

GANDHAGA PARPAM (wt:0.41210g)

Elements	Wavelength (nm)	Concentration
Al	396.152	BDL
As	188.979	BDL
Ca	315.807	10.180 mg/l
Cd	228.802	BDL
Cu	327.393	BDL
Fe	238.204	11.376 mg/l
Hg	253.652	BDL
K	766.491	03.821 mg/l
Mg	285.213	01.104 mg/l
Na	589.592	14.320 mg/l
Ni	231.604	BDL
Pb	220.353	BDL
P	213.617	186.34 mg/l
S	180.731	41.204 mg/l
Zn	206.200	3.210 mg/l

BDL: Below Detectable Limit (Normal-1ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

Toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic(As)	10ppm
Mercury(Hg)	1ppm
Lead (pb)	10ppm
Cadmium (Cd)	0.3ppm

INTERPRETATION

- This result indicates the presence of Calcium, Iron, Potassium, Sodium, Magnesium, Phosporus, Sulphur, Zinc
- **Calcium** is essential for maintaining the necessary level of bone mass. The body is constantly using calcium for the muscles and nerves. Maintenance of plasma calcium level within normal range is of vital importance because neuro-muscular excitability is dependent on plasma calcium level.
- **Phosphorous**, it decreases urine calcium, reduces demineralization of bone and increases calcium balance. It is an important constituent of phosphate buffers in the blood and urine. It is required for the formation of certain physiologically important phosphorus containing compounds like phospholipids, coenzymes and enzymes of intermediary metabolism. Contributes formation of ATP, ADP and creatine phosphate. [24]
- **Sodium and Potassium**: In the presence of Sodium and Potassium regulate the acid-base balance of the body fluids . They help to preserve the neuromuscular irritability by maintaining a state of equilibrium on account of their relative proportion in the ECF and ICF.
- **Magnesium** is a cofactor that regulates diverse biochemical reactions in the body, including protein synthesis, muscle, nerve function, blood glucose control and blood pressure regulation
- **Zinc** may be regarded as an antioxidant, protects the body against free radical damage and cell damage. Zinc is important for a healthy immune system. It enhances absorbtion of iron.It can produce healthy veins and arteries that enhance the blood circulation.
- **Sulphate** may prevent the occurrence of any infection, sulphate is potent anti oxidant activity in human body.
- **Ferrous Iron** Iron help to preserve many vital functions in the body, including general energy and focus, gastrointestinal processes, the immune system and regulation of body temperature.

XRD (X-Ray Diffraction):

X-Ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions.

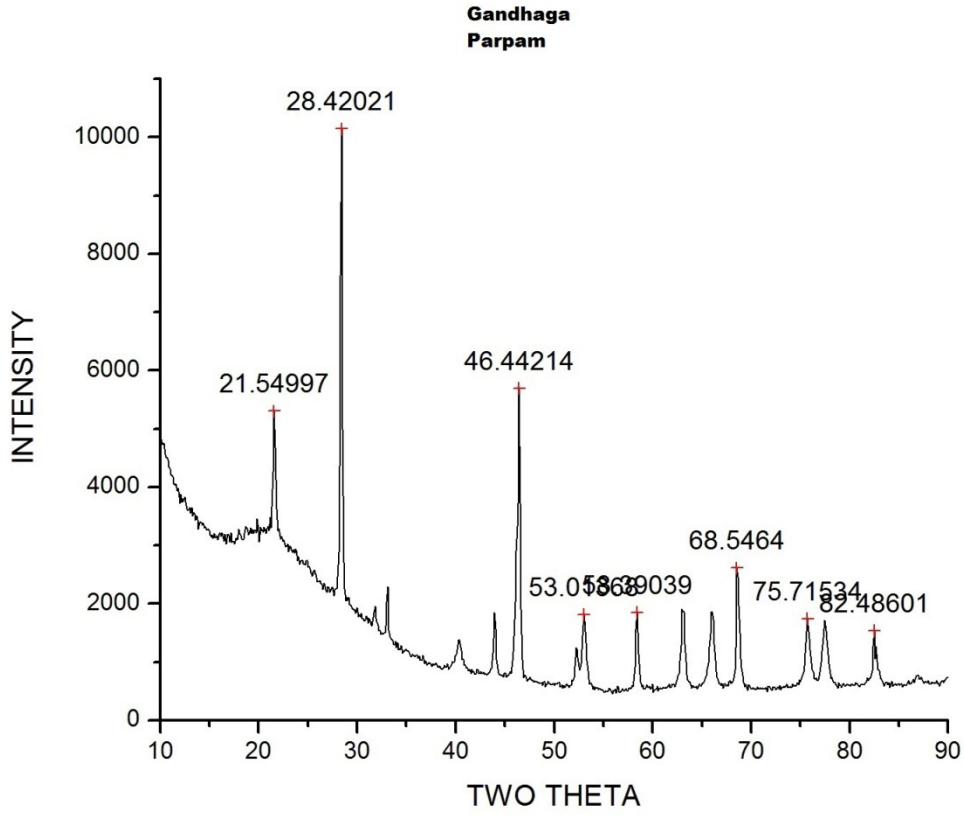


Fig. No. 8 XRD –Results of *GANDHAGA PARPAM*

This XRD finger print shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo-mineral formulations. The different peaks show the presence of minerals in the samples.

TOXICITY STUDIES

EVALUATION OF ACUTE TOXICITY STUDY OF

Effect of Acute Toxicity Study (14 Days) of *GANDHAGA PARPAM*

Table no –7 Physical and behavioral examinations.

Group no.	Dose(mg/kg)	Observation sign	No. of animal affected.
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Table no- 8 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table no- 9 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table no-10 Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

RESULT:

From acute toxicity study it was observed that the administration of ***GANDHAGA PARPAM*** at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of ***GANDHAGA PARPAM*** is 2000 mg/kg.

DISCUSSION

GANDHAGA PARPAM was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of ***GANDHAGA PARPAM*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.Food consumption of all treated animals was found normal as compared to normalgroup.

Body weight at weekly interval was measured to find out the effect of ***GANDHAGA PARPAM*** on the growth rate. Body weight change in drug treated animals was found normal.

INTERPRETATION:

GANDHAGA PARPAM was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of ***GANDHAGA PARPAM*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.Food consumption of all treated animals was found normal as compared to normalgroup.

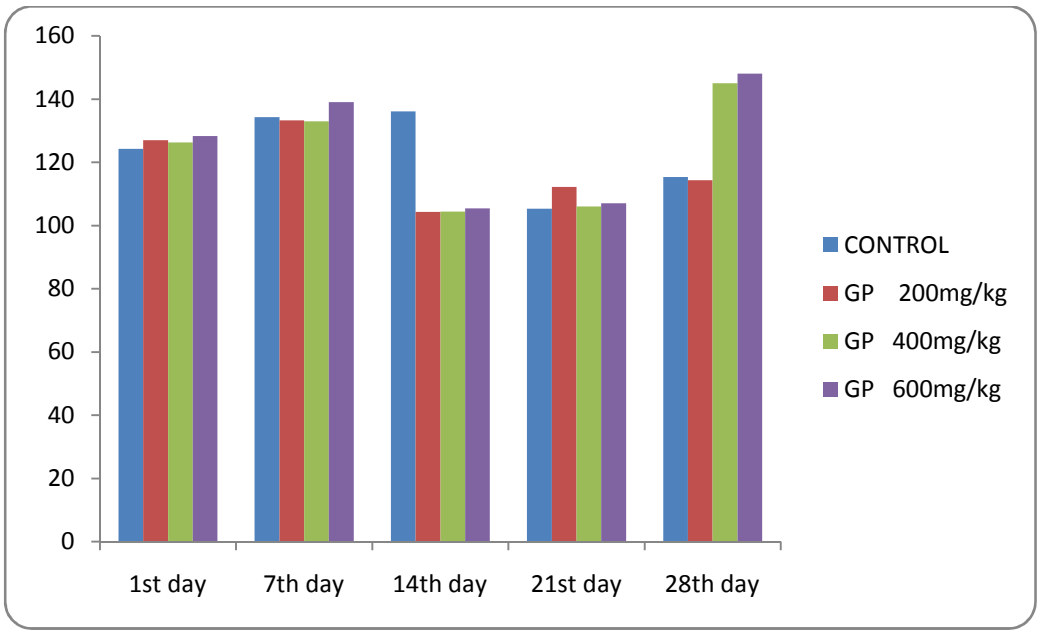
Body weight at weekly interval was measured to find out the effect of ***GANDHAGA PARPAM*** on the growth rate. Body weight change in drug treated animals was found normal.

SUB-ACUTE TOXICITY STUDY IN WISTAR RATS TO EVALUATE TOXICITY PROFILE OF *GANDHAGA PARPAM*

Table :11 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *GANDHAGA PARPAM ON* BODY WEIGHT IN GRAM

GROUP	CONTROL	LOW	MID	HIGH
1st day	124.3±1.03	127±1.543	126.3±2.231	128.3±2.23
7th day	134.3±1.03	133.3±1.343	133±2.113	139±2.11
14th day	136.1±1.004	104.3±1.12	104.4±2.012	105.4±2.012
21st day	105.3±2.120	112.2±1.501	106±1.131	107±1.13
28th day	115.3±1.041	114.3±1.202	145±2.0405	148±2.040

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

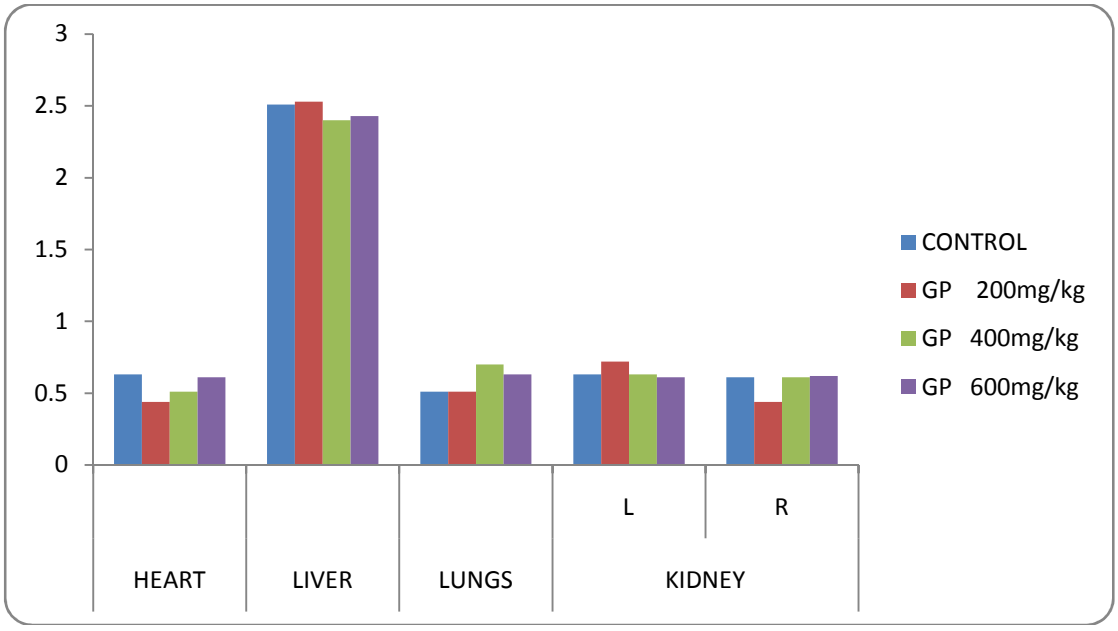


EFFECT OF SUBACUTE DOSE (28 DAYS) OF *GANDHAGA PARPAM*

Table : 12 *GANDHAGA PARPAM* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP		CONTROL	LOW	MID	HIGH
HEART		0.63±0.02	0.44±0.04	.51±0.11	0.61±0.02
LIVER		2.51± 0.23	2.53±0.23	2.40±0.01	2.43± 0.23
LUNGS		.51±0.10	0.51±0.14	0.70±0.24	.63±0.10
KIDNEY	L	0.63±0.02	0.72±0.03	0.63±0.02	0.61±0.02
	R	0.61±0.024	0.44±0.02	0.61±0.24	0.62±0.24

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

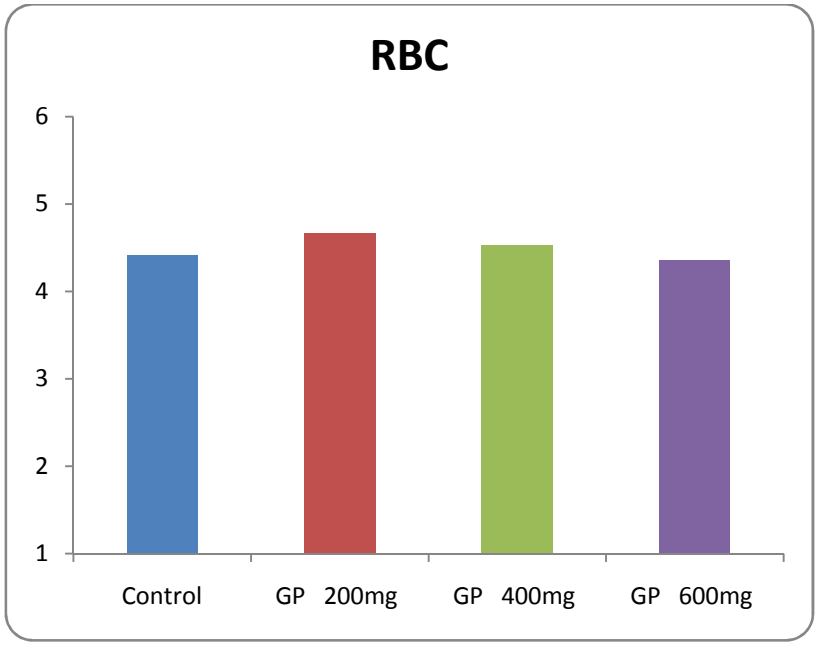


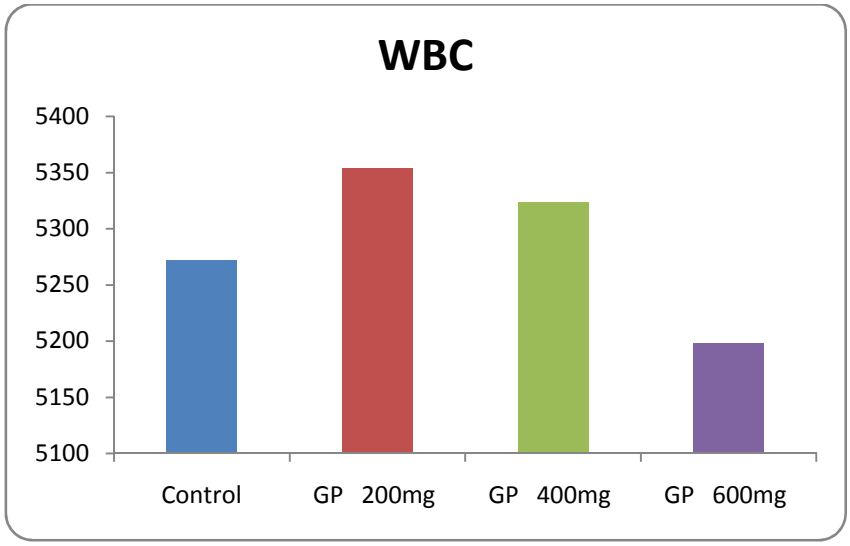
EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *GANDHAGA PARPAM* ON HAEMATOLOGICAL PARAMETERS

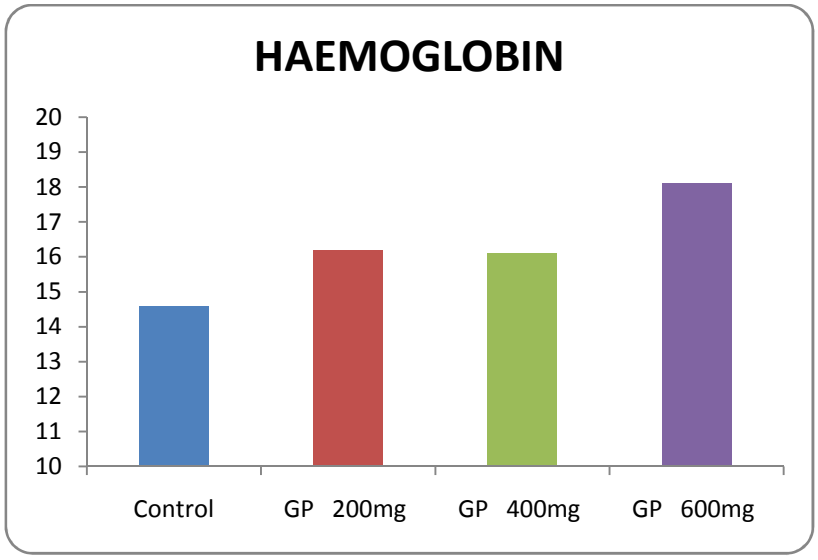
Table no 13

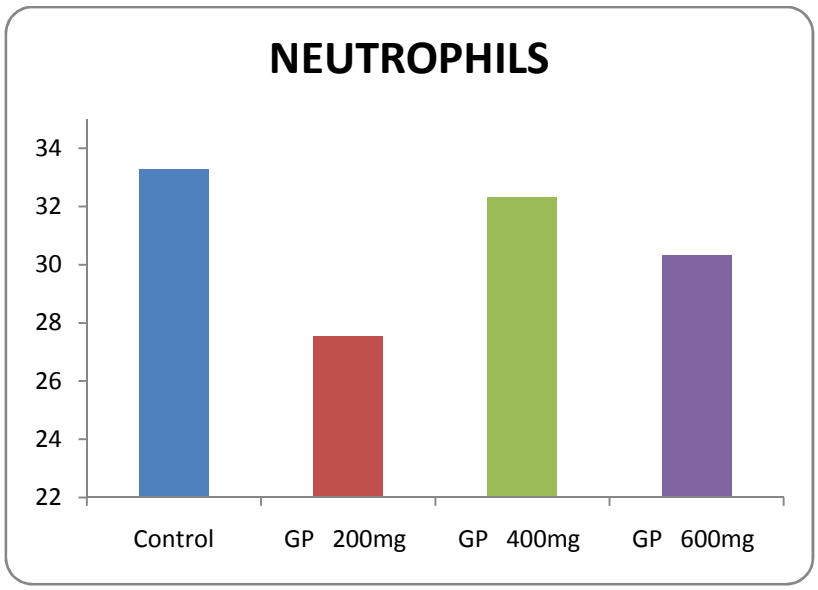
Drug treatment	RBC million cells/cmm	WBC cells/cmm	Haemoglobin gm %	Differential count %			
				Neutrophils	Eosinophils	Monocyte	Lymphocyte
Control	4.41±0.40	5272.41±23.32	14.60±0.45	33.27±1.20	4.73±0.11	0.65±0.15	25.13±3.32
LOW	4.67±0.20	5354.04±23.22	16.20±0.43	27.54±1.41	2.30±0.14	0.42±0.30	25.22±3.51
MID	4.53±0.21	5324.25±32.35	16.11±1.03	32.32±2.22	3.64±0.12	0.4052±0.40	25.13±3.32
HIGH	4.36±0.21	5198.25±32.35	18.11±1.03	30.32±2.22	3.70±0.12	0.54±0.40	26.13±3.32

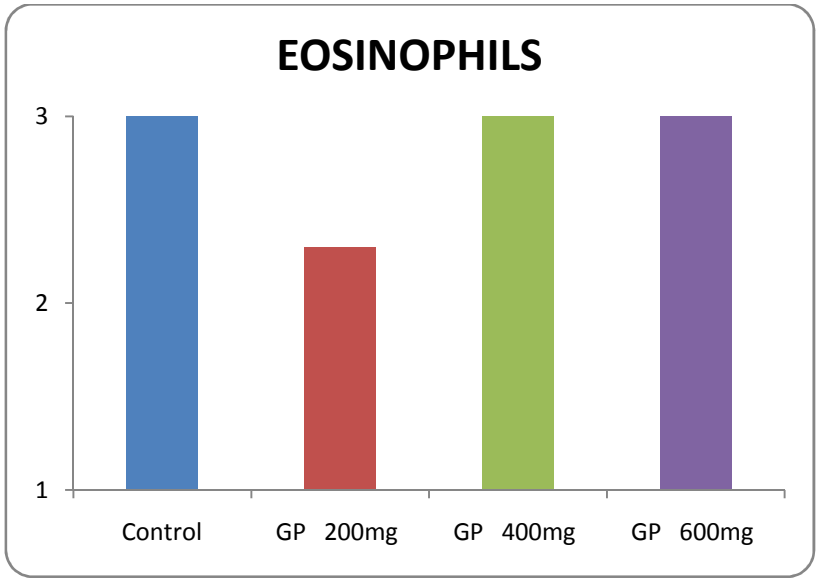
Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

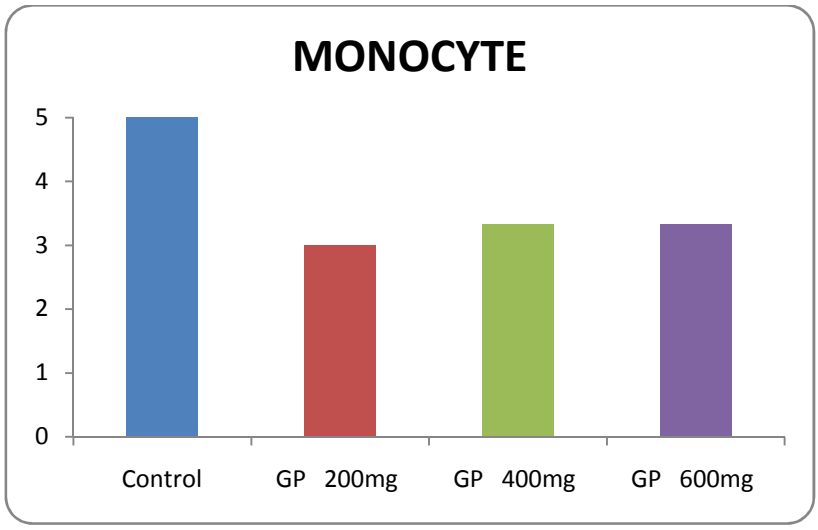



WBC
5400
5350
5300
5250
5200
5150
5100
Control
GP 200mg
GP 400mg
GP 600mg


HAEMOGLOBIN
20
19
18
17
16
15
14
13
12
11
10
Control
GP 200mg
GP 400mg
GP 600mg


NEUTROPHILS
34
32
30
28
26
24
22
Control
GP 200mg
GP 400mg
GP 600mg


EOSINOPHILS
3
2
1
Control
GP 200mg
GP 400mg
GP 600mg


MONOCYTE
5
4
3
2
1
0
Control
GP 200mg
GP 400mg
GP 600mg

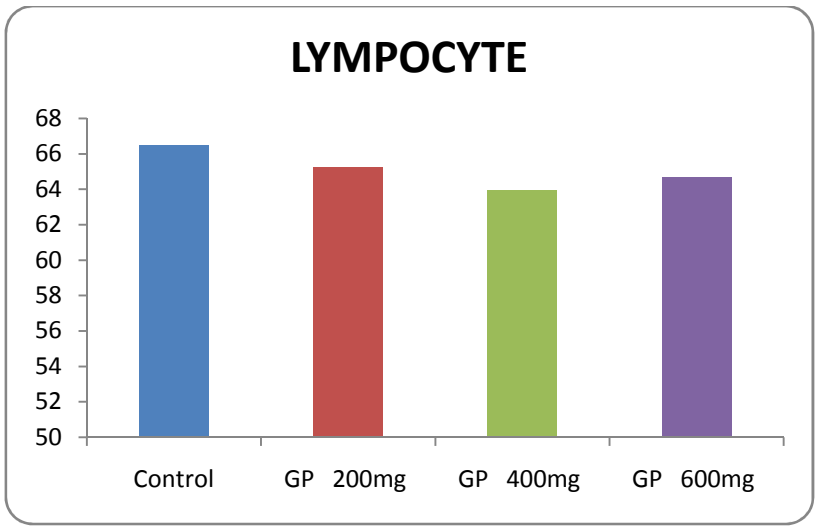
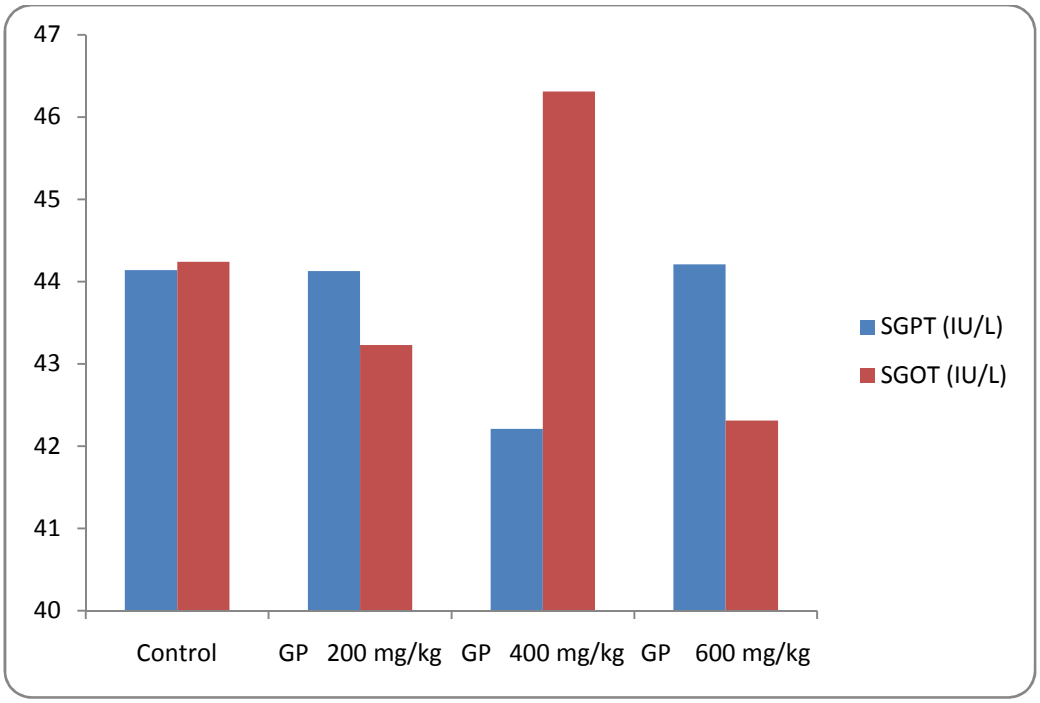
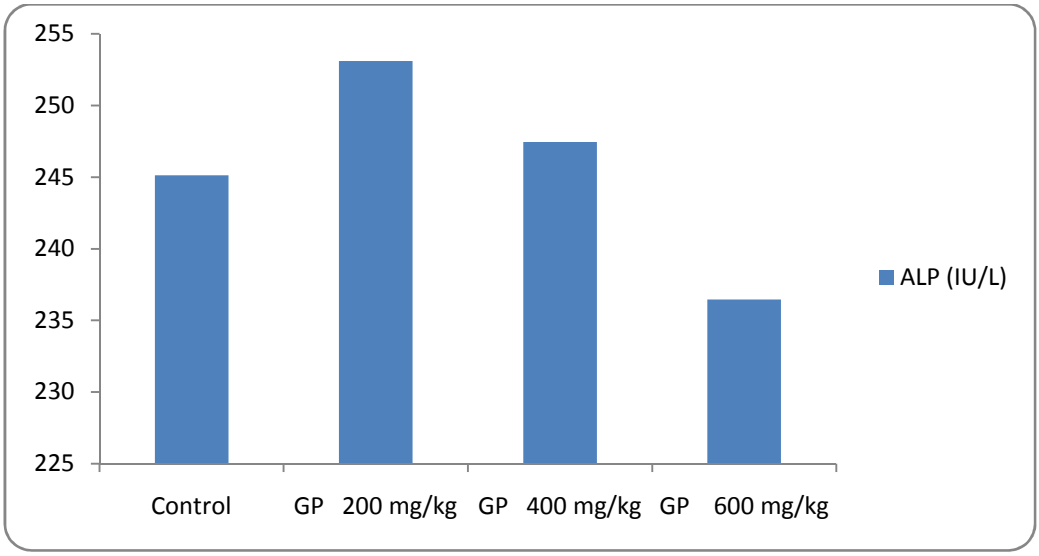
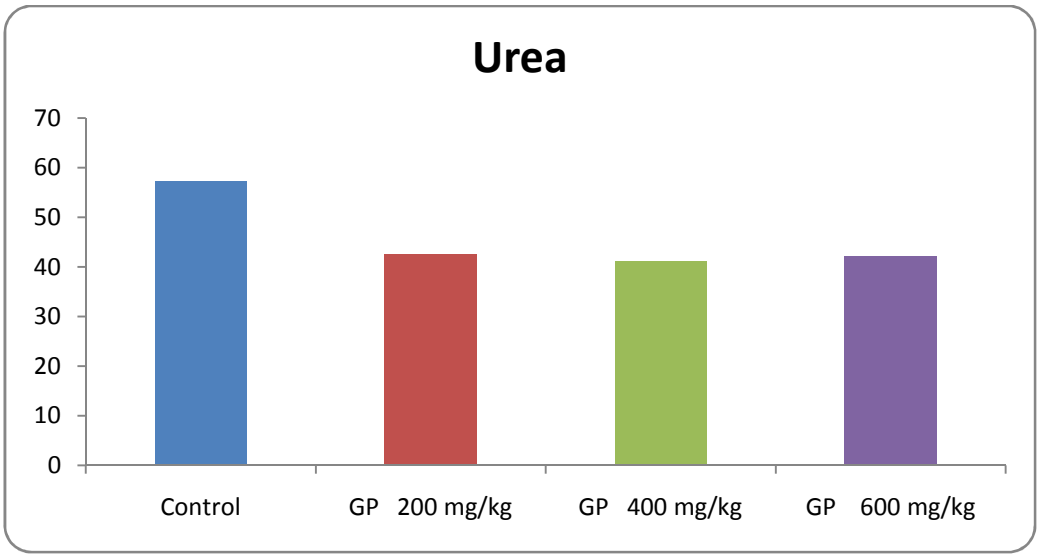

LYMPOCYTE
68
66
64
62
60
58
56
54
52
50
Control
GP 200mg
GP 400mg
GP 600mg

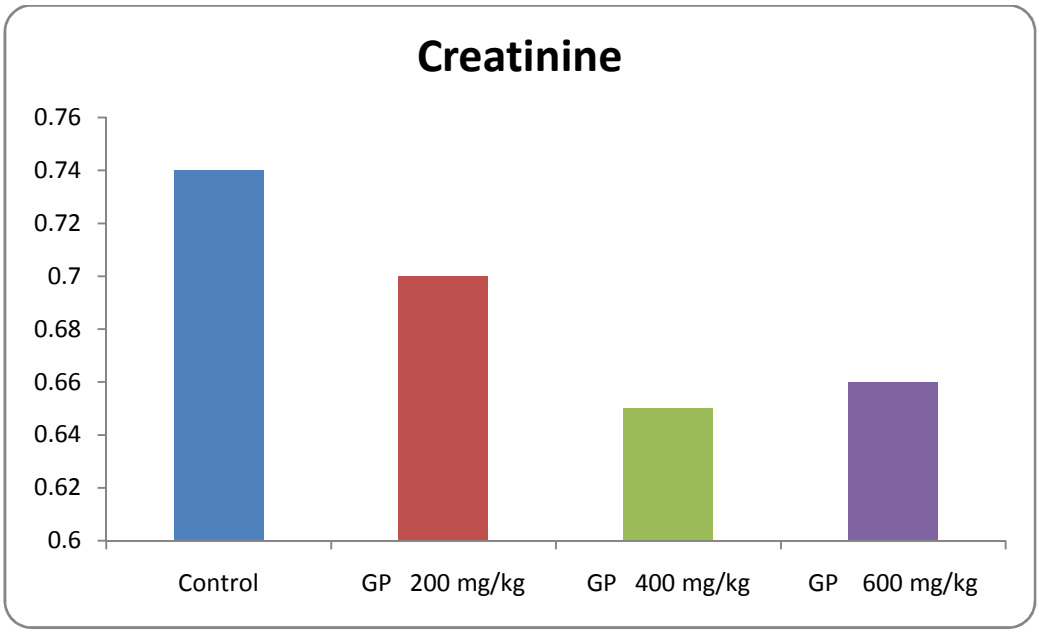
Table : 14 EFFECT OF SUB- ACUTE DOSE(28 DAYS)OF *GANDHAGA PARPAM* ON BIOCHEMICAL PARAMETERS

Drug Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	44.14±3.0	44.24±4.31	245.12±11.32	57.35±3.00	0.74±0.03
LOW	44.13±3.22	43.23±4.01	253.11±12.42	42.53±2.42	0.70±0.04
MID	42.21±4.44	46.31±2.21	247.45±4.14	41.12±2.22	0.65±0.04
HIGH	44.21±4.44	42.31±2.21	236.45±4.14	42.12±2.22	0.66±0.04




255
250
245
240
235
230
225
ALP (IU/L)
Control
GP 200 mg/kg
GP 400 mg/kg
GP 600 mg/kg


Urea
70
60
50
40
30
20
10
0
Control
GP 200 mg/kg
GP 400 mg/kg
GP 600 mg/kg


Creatinine
0.76
0.74
0.72
0.7
0.68
0.66
0.64
0.62
0.6
Control
GP 200 mg/kg
GP 400 mg/kg
GP 600 mg/kg

EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF*G P*BIOCHEMICAL PARAMETERS

GROUP	CONTROL	G P (200mg/kg)	G P (400mg/kg)	G P (600mg/kg)
TOTAL BILIRUBIN (mg/dl)	0.508±0.2457	0.658±0.27	0.8198±0.76	0.64±0.9

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

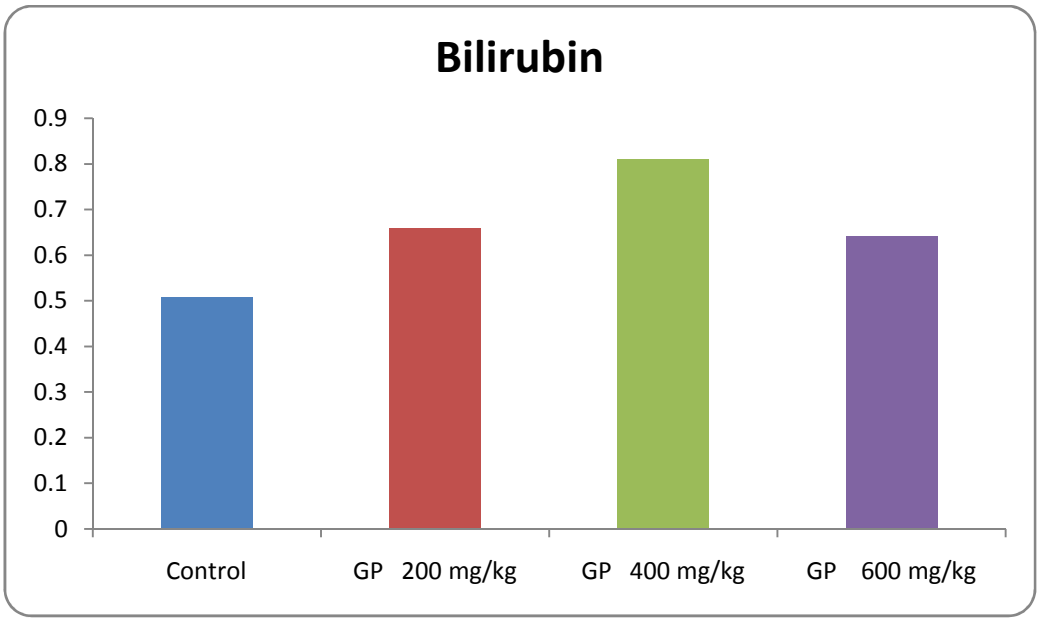


Table: 15 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *GANDHAGA PARPAM* ON FOOD INTAKE IN GRAM

GROUP	CONTROL	L	M	H
1st DAY	21.33±13.6110	22.1672±15.3	15.10±22.71	20.5±8.62
7th DAY	18.5±12.	13.863±13.67	19.73±10.853	14.17±15.41
14th DAY	21.83±8.72	13.83±15.28	13±14.96	22.72±9.981
21st DAY	14.87±13.4	18±8.466	18.88±10.43	22.17±9.02
28th DAY	15.10±11.38	21.38±11.50	13±8.90	13±7.57

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

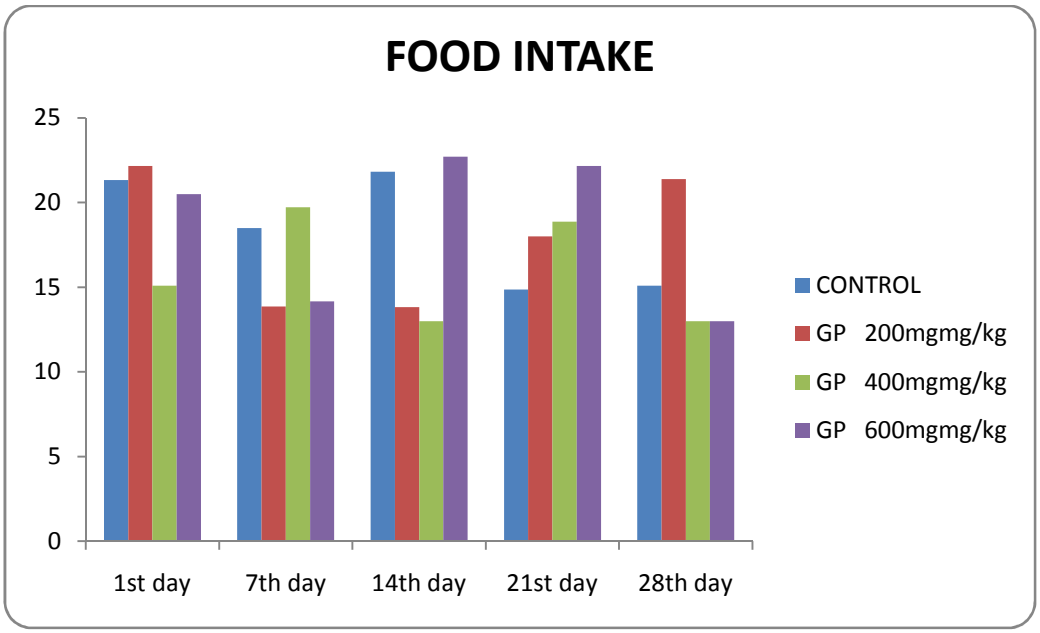


Table: 16 Effect of Sub- Acute Dose (28 Days) Of *GANDHAGA PARPAM* On Water Intake in ml

GROUP	CONTROL	GP (200mg/kg)	GP (400mg/kg)	GP (600mg/kg)
1st DAY	98.38±13.5110	89.1672±14.26	102.10±21.99	67.5±7.03
7th DAY	85.5±11.7938	100.63±12.60	76.6673±9.63	81.6717±14.40
14th DAY	58.3383±8.72817	90.83±14.22	80±13.92	89.1672±8.81
21st DAY	91.6687±12.4949	85±8.62	65.88±9.430	89.1717±8.02
28th DAY	82.10±11.3840	88.3348±11.5004	80±8.90061	70±7.57773

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group

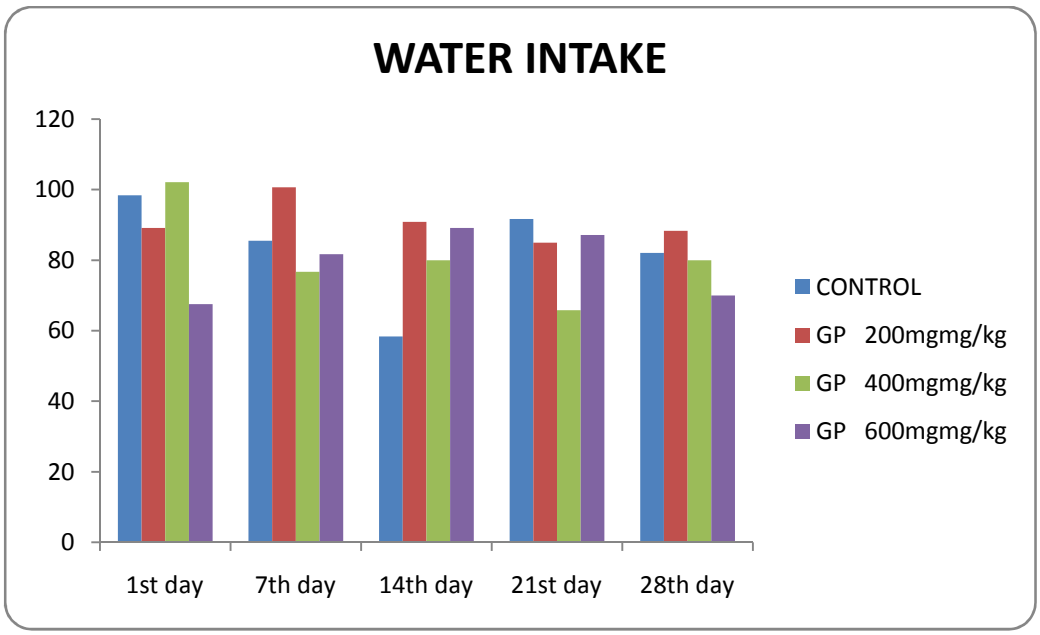
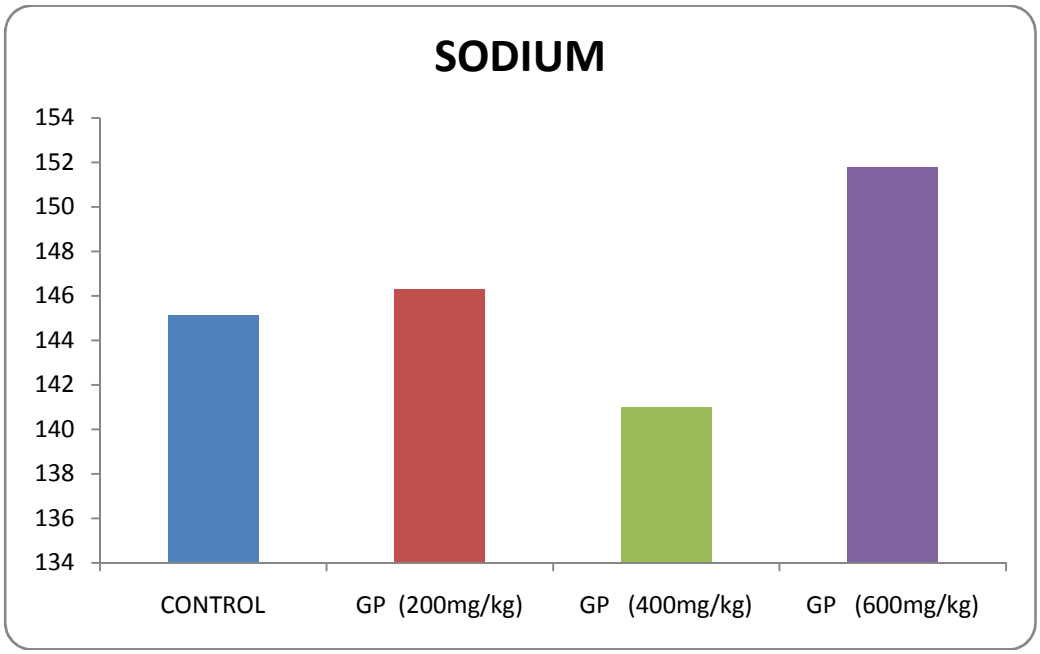
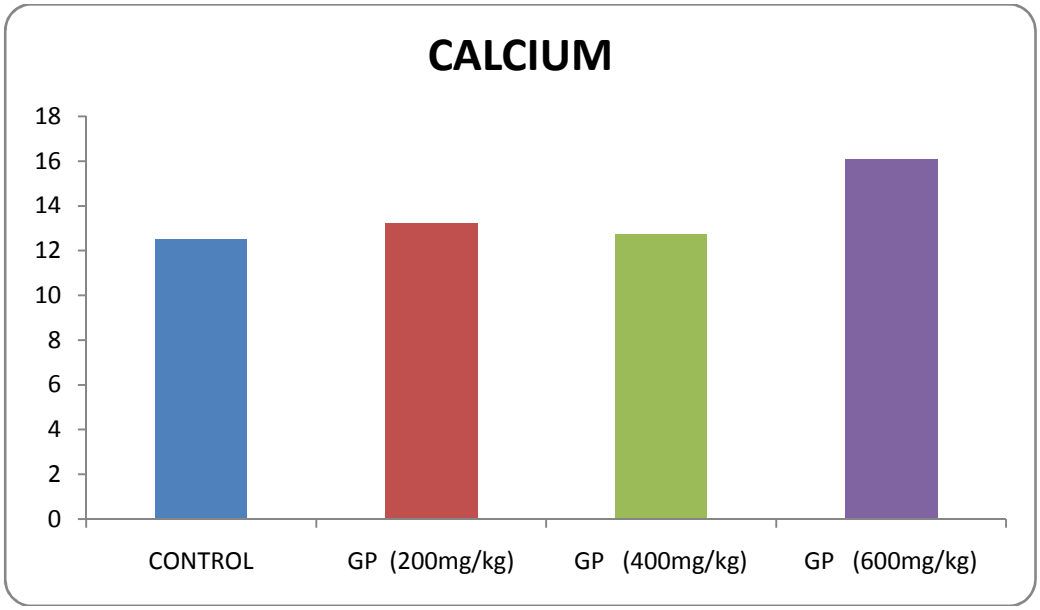


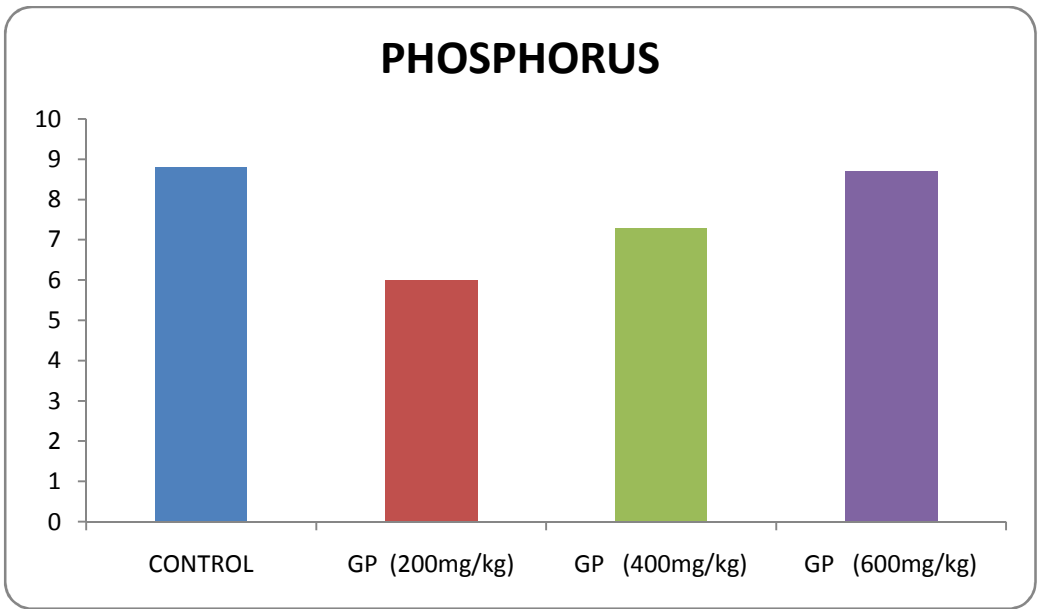
Table: 17 EFFECT OF SUB ACUTE DOSES (28 DAY) OF *GANDHAGA PARPAM* ON ELECTROLYTES: -

GROUP	CONTROL	GP (200mg/kg)	GP (400mg/kg)	GP (600mg/kg)
Sodium (mg/dl)	145.10±0.55	146.30±0.62	141±0.1	151.80±0.70
Calcium(mg/dl)	12.50±0.189	13.20±0.83***	12.7±0.1***	16.10±0.11***
Phosphorus (U/L)	8.8±0.017	6.0±0.019915^{ns}	7.30±0.01^{ns}	8.7±0.32*

Values are expressed as mean ± SEM Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); NS- non-significant, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.




CALCIUM
18
16
14
12
10
8
6
4
2
0
CONTROL
GP (200mg/kg)
GP (400mg/kg)
GP (600mg/kg)


PHOSPHORUS
10
9
8
7
6
5
4
3
2
1
0
CONTROL
GP (200mg/kg)
GP (400mg/kg)
GP (600mg/kg)

RESULTS:

CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.12 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on the day 29th and recorded in Table no 14 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

INTERPRETATION:

1) All the animals from control and all the treated dose groups up to 15ml/kg survived throughout the dosing period of 28 days.

2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.

3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days

5) Haematological analysis conducted at the end of the dosing period on day 29th, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29^{th}, no abnormalities attributable to the treatment.

7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

PHARMACOLOGICAL RESULTS

ANTI CANCEROUS ACTIVITY OF SIDDHA PREPARATION *GANDHAGA PARPAM ON* DALTON'S LYMPHOMA ASCITES IN MICE

Table No.18 -Effect of *GANDHAGA PARPAM* on Hematological parameters

TREATMENT	Total WBC Cells /mlx10^3	Rbc Count Mill/cumm	Hb Gm/dl	PCV %	Platelets Lakhs/cumm
G1	9.97 ±3.30	5.49±2.99	14.44 ±4.44	36.67±4.86	5.22±2.81
G2	15.17 ±4.67^{a**}	4.54±2.50^{a**}	6.34 ±2.82^{a**}	38.47±5.57^{a**}	3.62±2.64^{a**}
G3	9.57 ±3.94^{b**}	5.97±2.87^{b**}	12.6±3.82^{b**}	21.23±4.62^{b**}	4.69±2.56^{b**}
G4	12.57±4.97^{b*}	4.46±2.66^{b*}	12.92±3.26^{b*}	20.47±4.97^{b*}	5.12±2.53^{b*}
G5	10.62 ±4.54^{b*}	4.50±2.84^{b*}	12.82±3.80^{b*}	26.26±3.62^{b*}	5.34 ±2.76^{b*}

G_1 – Normal Control, G_2 – Cancer Control, G_3 – Positive control, G_4 – Treatment control (MEA), G5 – Treatment control (MEC)

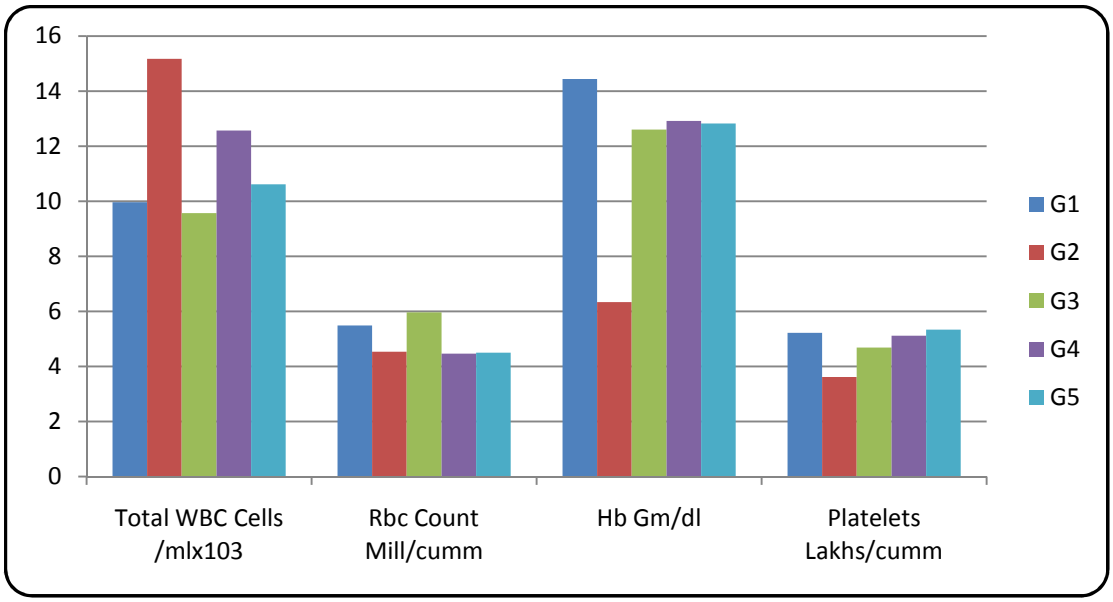
All values are expressed as mean ± SEM for 6 animals in each group.

**a – Values are significantly different from Normal control (G_1) at $P < 0.001$

*b – Values are significantly different from cancer control (G_2) at $P < 0.01$

**b – Values are significantly different from cancer control (G_2) at $P < 0.001$

Fig. No.9 -Effect of Effect of *GANDHAGA PARPAM* on Hematological parameters



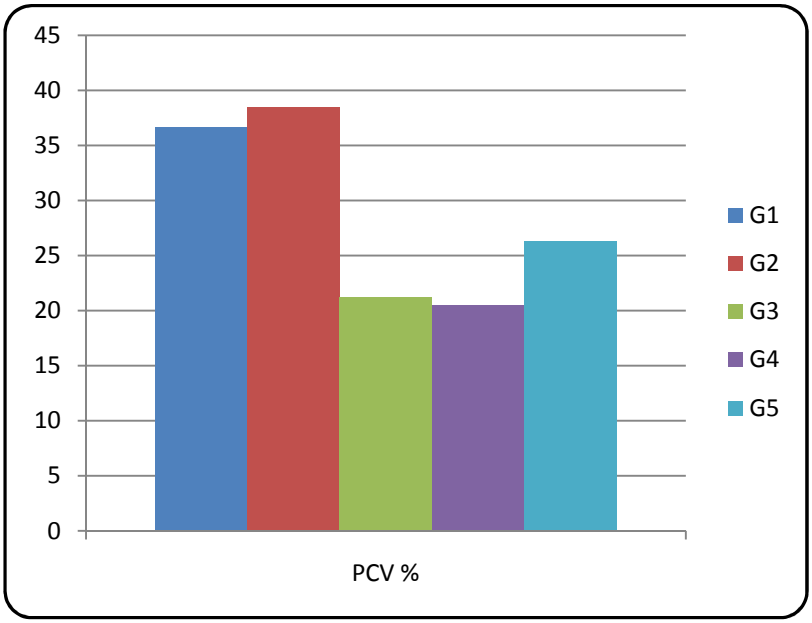


Table No.19

Effect of *GANDHAGA PARPAM* on serum Enzymes and lipid proteins

Treatment	Cholesterol (mg/dl)	TGL (mg /dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
G_1	98.32±5.67	127.6±6.52	36.46 ±3.32	34.42 ±3.34	130.37 ±4.32
G_2	144.97±6.62^{a**}	206.47±8.42^{a**}	88.47±4.82^{a**}	58.22±4.27^{a**}	242.52±6.30^{a**}
G_3	112.52±5.94^{b**}	152.57±5.82^{b**}	56.27 ±3.82^{b**}	44.42±3.72^{b**}	167.32±4.42^{b**}
G_4	127.27±5.42^{b*}	167.52±4.24^{b*}	75.24 ±4.34^{b*}	48.56±3.80^{b*}	196.37±5.42^{b*}
G_5	124.32±4.57^{b*}	165.24±4.42^{b*}	74.42±3.94^{b*}	47.52 ±3.27^{b*}	190.67±4.67^{b*}

All values are expressed as mean ± SEM for 6 animals in each group.

G_1 – Normal Control, G2 –Cancer Control, G3 –Positive control, G_4 –Treatment control (MEA), G_5– Treatment control (MEC).

All values are expressed as mean ± SEM for 6 animals in each group.

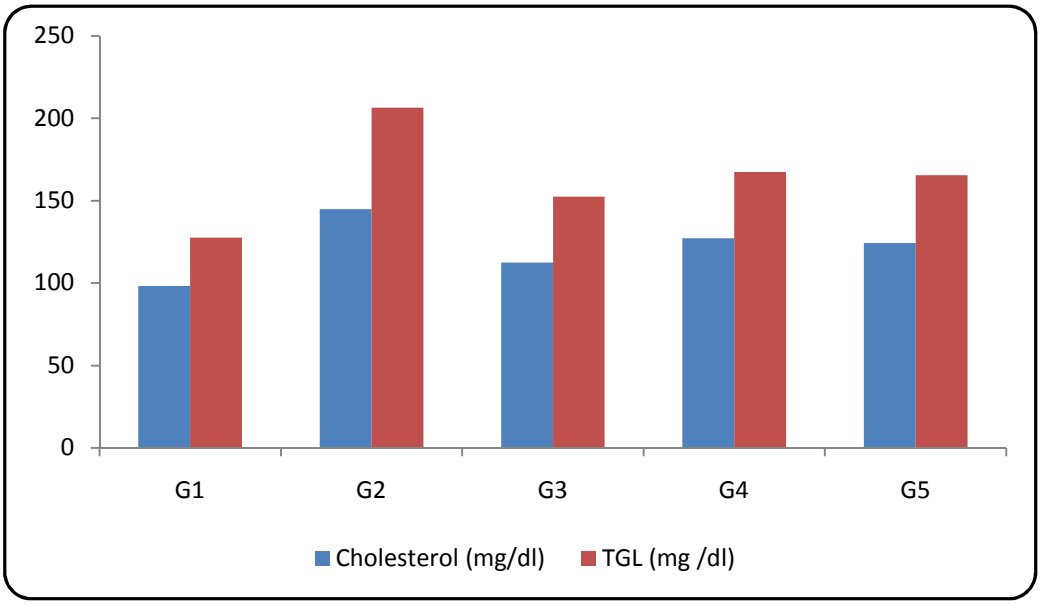
**a – Values are significantly different from control (G_1) at $P < 0.001$

*b – Values are significantly different from cancer control (G_2) at $P < 0.01$

**b – Values are significantly different from cancer control (G_2) at $P < 0.001$

Fig. No. 10

Effect of *GANDHAGA PARPAM* on serum Enzymes and lipid proteins



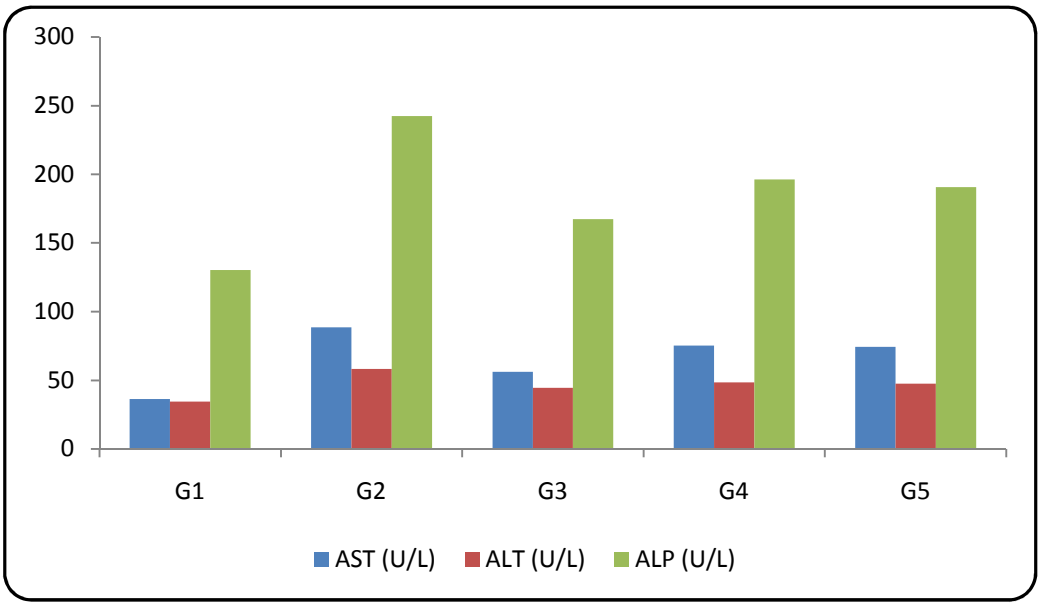


Table No.20

Effect of *GANDHAGA PARPAM* on the life span, body weight and cancer cell count of tumor induced mice.

Treatment	Number of animals	% ILS Life span	Increase in Body weight grams	Cancer cell count ml X 10^6
G_1	6	>30 days	5.22±2.52	-
G_2	6	48%	10.86±3.12^{a**}	5.62±2.34^{a**}
G_3	6	88%	6.74±2.57^{b**}	4.41±2.33^{b**}
G_4	6	68%	7.58±2.82^{b*}	5.17±2.49^{b*}
G_5	6	66%	7.15±2.92^{b*}	4.10±2.42^{b*}

All values are expressed as mean ± SEM for 6 animals in each group.

G_1 – Normal Control, G2 –Cancer Control, G3 –Positive control, G_4 –Treatment control (MEA), G_5– Treatment control (MEC).

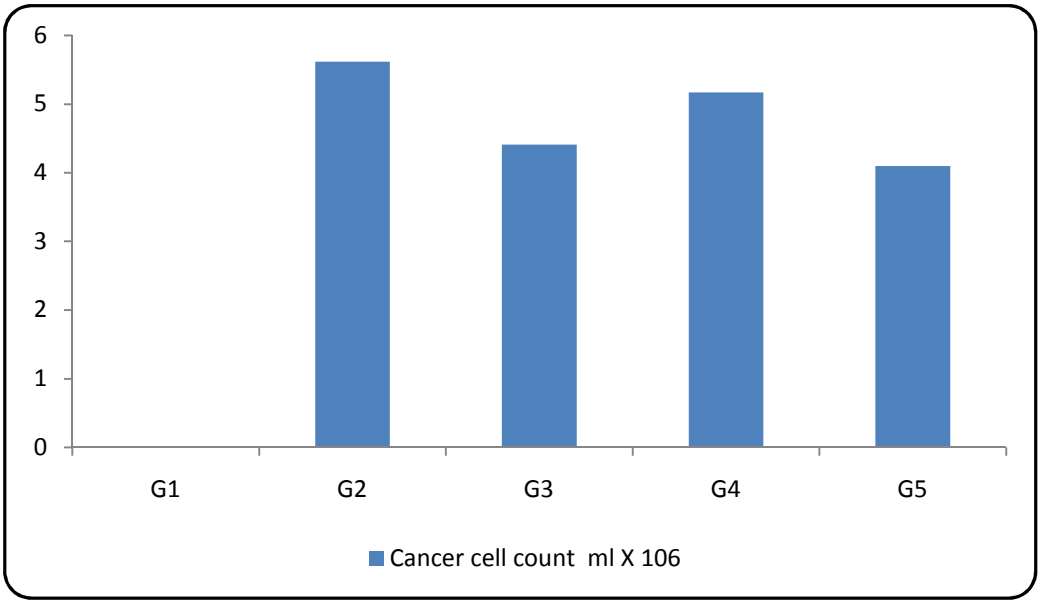
**a – Values are significantly different from normal control (G_1) at $P < 0.001$

*b – Values are significantly different from cancer control (G_2) at $P < 0.01$

**b – Values are significantly different from cancer control (G_2) at $P < 0.001$

Fig . No. 11

Effect of *GANDHAGA PARPAM* on the life span, body weight and cancer cell count of tumor induced mice.



INTERPRETATION :

In DLA tumor bearing, a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. Treatment with *GANDHAGA PARPAM* inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the lifespan of animals. It may be concluded that *GANDHAGA PARPAM* by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DLA bearing mice. Thus *GANDHAGA PARPAM* have anticancer activity against DLA bearing mice.

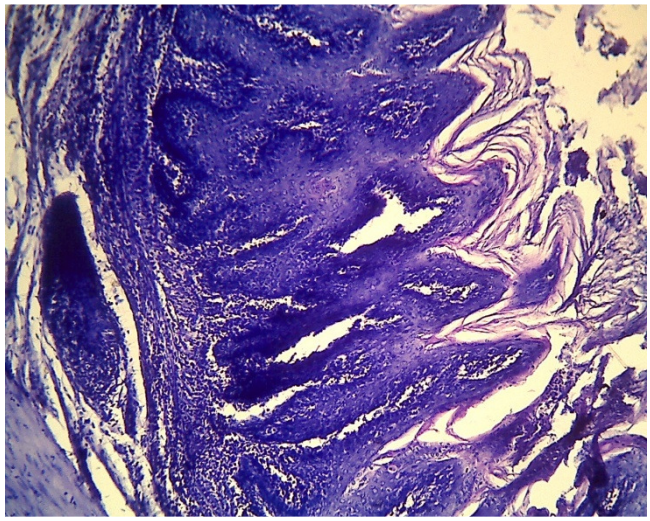
Usually, in cancer chemotherapy the major problems that are being encountered are of myelo suppression and anemia. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. Treatment with *GANDHAGA PARPAM* brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels significantly. This clearly indicates that *GANDHAGA PARPAM* possess protective action on the haemopoietic system.

It was reported that the presence of tumor in the human body or in the experimental animals is known to affect may function of the liver. The significantly elevated level of total **cholesterol**, TG, AST, ALT, ALP in serum of tumor inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by *GANDHAGA PARPAM* treatments.

In the present study, the biochemical examination of DLA inoculated animals showed marked changes indicating the toxic effect of the tumor. The normalization of these effects observed in the serum treated with *GANDHAGA PARPAM* supported the potent antitumor and hepatoprotective effect of the *GANDHAGA PARPAM*

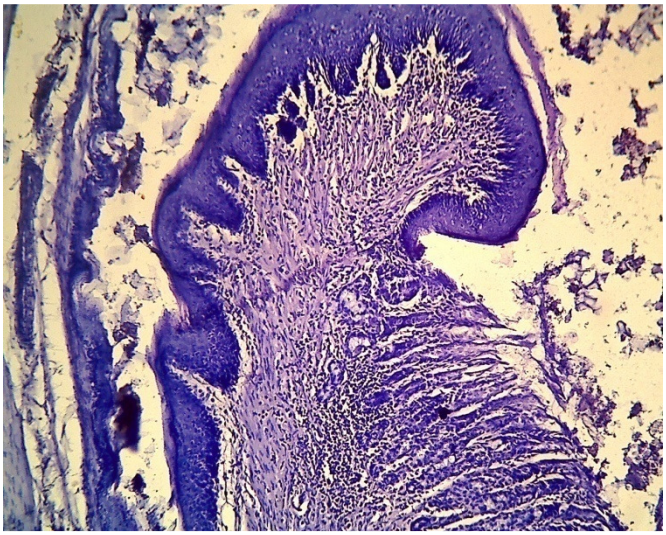
Fig. No. 12 HISTOPATHOLOGY

Figure No. 1 – NORMAL CONTROL (UTERUS.)



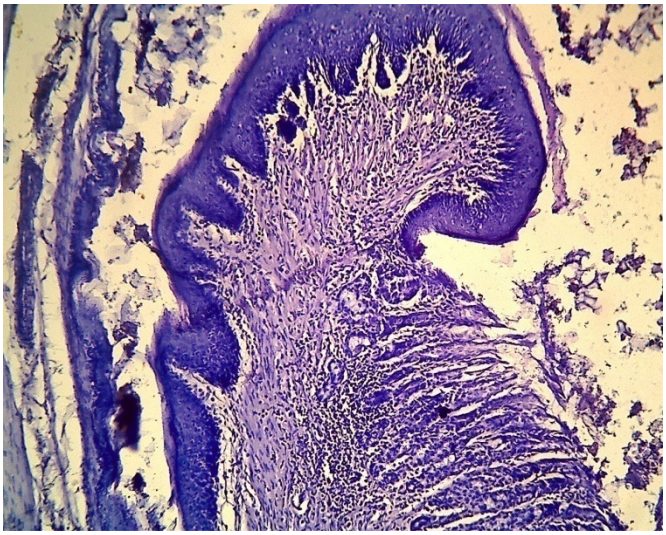
SECTION SHOW STRUCTURE OF UTERUS WITH CERVIX APPEAR NORMAL

Figure No.2 – TUMOR CONTROL (UTERUS.)



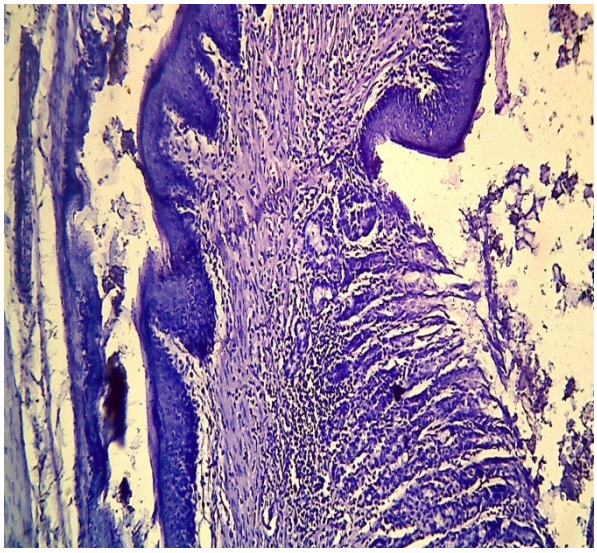
SECTION SHOW STRUCTURE OF UTERUS WITH CERVIX CANCER

Figure No. 3 – STANDARD CONTROL (UTERUS.)



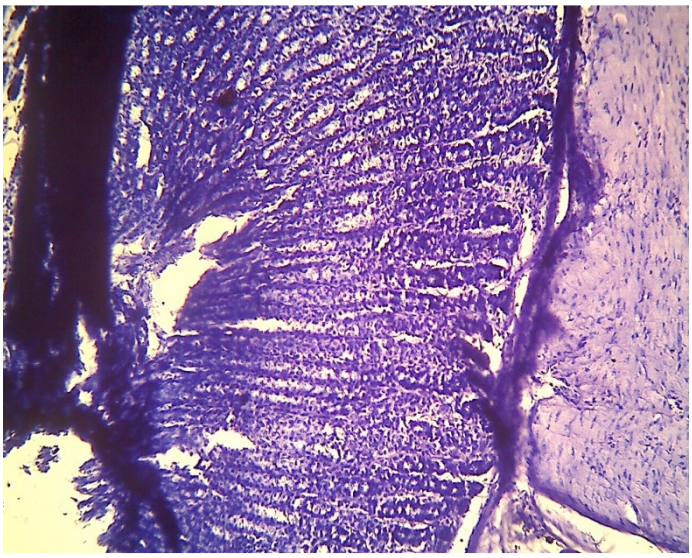
SECTION SHOW STRUCTURE OF UTERUS WITH NO CERVIX CANCER

Figure No.4 – TREATED WITH ld (UTERUS)



SECTION SHOW STRUCTURE OF UTERUS WITH NO CERVIX CANCER

Figure No.5 – TREATED WITH hd (LIVER)



SECTION SHOW STRUCTURE OF UTERUS WITH NO CERVIX CANCER

ANTI-OXIDANT ACTIVITY OF SIDDHA PREPARATION *GANDHAGA PARPAM* BY DPPH RADICAL SCAVENGING ASSAY

Table No.21. Anti-oxidant activity of *GANDHAGA PARPAM*

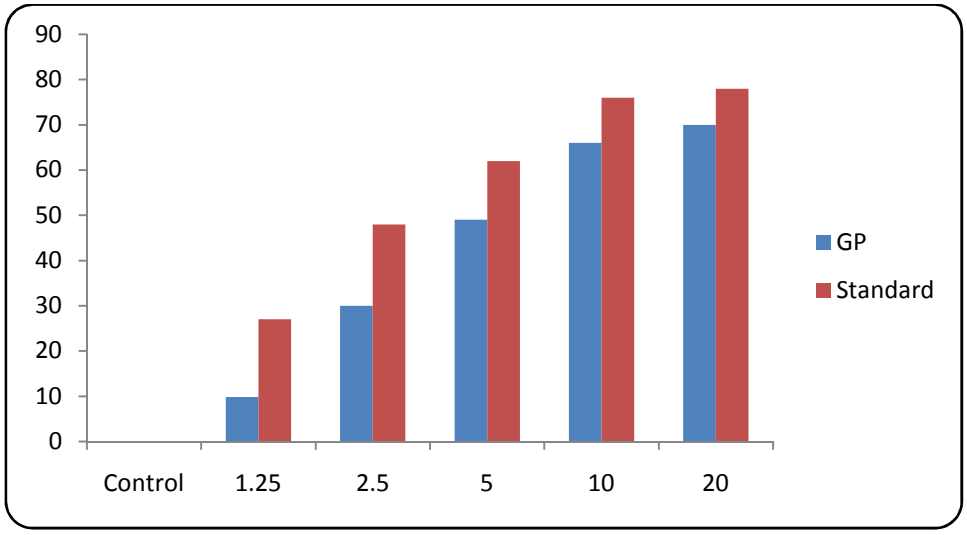
Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	GP	Standard	GP	Standard
Control	0.5461	0.354	-	-
1.25	0.4921	0.262	9.8	27
2.50	0.3825	0.182	30	48
5	0.2782	0.104	49	62
10	0.1820	0.082	66	76
20	0.1025	0.076	70	78

µg/ml: microgram per millilitre.

Drug: GANDHAGA PARPAM (1.25-20µg/ml). Standard: BHT (10mg/ml)

IC 50 value of GP = 10 *µg/ml* IC 50 value of STD = 5 *µg/ml*

Fig. No. 13. Anti-oxidant activity of *GANDHAGA PARPAM*



INTERPRETATION:

From the investigation of DPPH radical scavenging assay of *GANDHAGA PARPAM* it was concluded that the test drug has shown promising antioxidant activity and exhibits significant percentage inhibition against DPPH radicals when compared to that of standard BHT. Because of this high antioxidant therapeutic nature the drug *GANDHAGA PARPAM* will helps to treat cancer. Antioxidants play a major role in the treatment of cancer.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF GANTHAGA PARPAM BY CARRAGEENAN INDUCED HINDPAW OEDEMA METHOD

TABLE No. 22 Anti-inflammatory activity of *Gandhaga Parpam*

Serial no	Name of drugs/groups	Dose/100 gram body weight	Initial reading average	final reading average	Mean difference	Percentage inflammation	Percentage inhibition
1	Water	2 ml	0.9	1.2		100	-
2	Diclofenac	5 mg/kg	0.9	0.8	0.2	11.1	88.9
3	GP	100mg/kg	0.8	1.1	0.30	37.5	62.5
4	GP	200mg/kg	0.8	0.7	0.1	16.4	74.6

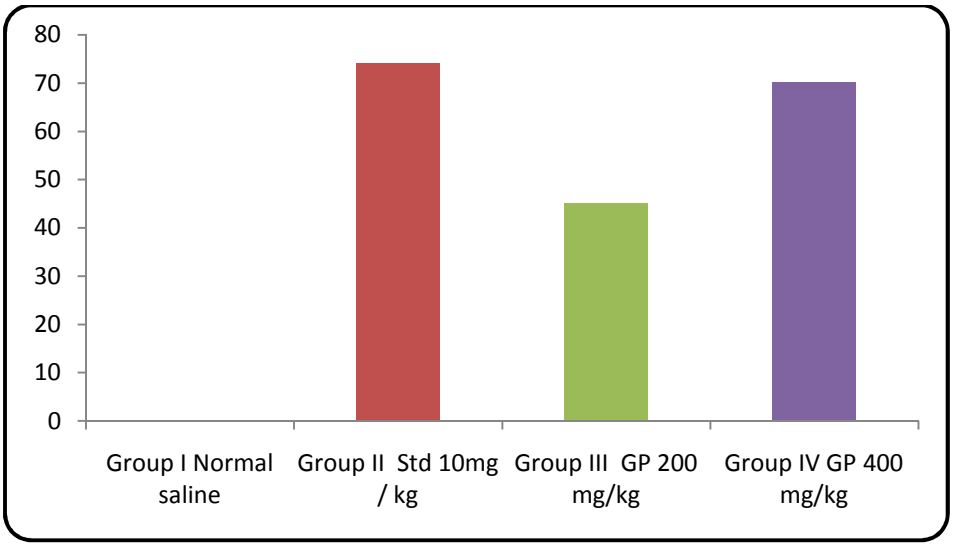
* Data are expressed as Mean ± S.E.M.

*Data were analyzed by one way ANOVA followed by Newman's keul's multiple range tests, to determine the significance of the difference between the control group and rats treated with the test compounds.

*a Values were significantly different from normal control at P< 0.01.

Fig.No. 14

Evaluation of Anti-Inflammatory



INTERPRETATION:

GANTHAGA PARPAM at doses100mg/kg& 200mg/kg were tested for their Anti- inflammatory activity by using carrageenan Induced rat paw edema method and the results are tabulated in table no22. The results reveals that **GANTHAGA PARPAM** at 100mg/kg & 200mg/kg doses possesses significant Anti- inflammatory activity when compared to control group at p<0.01.

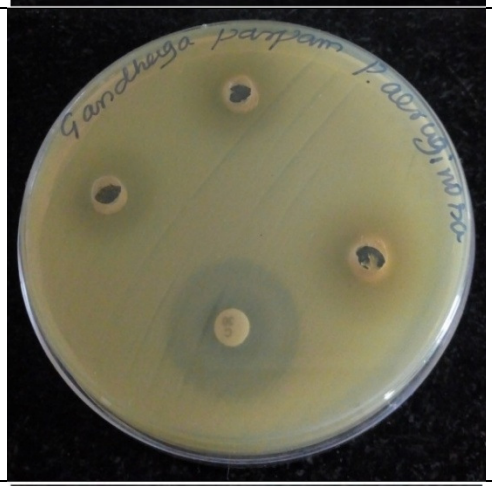
ANTIMICROBIAL ACTIVITIES OF *GANDHAGA PARPAM* BY AGAR WELL DIFFUSION METHOD

Table. No. 23

S.No.	Test Pathogens	Result	Zone of Inhibition (mm) at 30µl	
			Positive Control (Chloromphenical)	Size of Inhibition
1.	*Escherichia coli*	Sensitive	25 mm	20 mm
2	*Staphylococcus aureus*	Sensitive	20 mm	10 mm
3	*Klebsiella pneumoniae*	Sensitive	20 mm	10 mm
4	*Pseudomonas aeruginosa*	Resistant	23 mm	5 mm
5.	*Candida sp (Ketaconozole)*	Sensitive	24 mm	8 mm

Fig. No. 15
Antimicrobial activity result

S.No.	Bacterial Pathogens	Plates
1.	*Escherichia coli*	
2.	*Staphylococcu aureus*	

3.	*Klebsiella sps.*	
4.	*Pseudomonas aeruginosa*	
5.	*Candida sp*	

INTERPRETATION :

Both gram positive, gram negative bacteria E.Coli, Klebsiella, Pneumonia and Staphylococcus aureus and fungus Candida species were found to be sensitive when compared to the standard drug Chloromphenica and Ketaconozole. The bacteria Pseudomona aeruginosa were found to be resistance. The mineral drug *GANDHAGA PARPAM* exhibited broad spectrum activity against bacterial and fungal pathogenes at 100 mg/ml concentration of the drug.

7. SUMMARY

The test drug Ganthaga Parpam is selected from the text Agasthiyar Vaithiya Vallathy 600,Pg.No.218&219 Authored by Dr.K.Velusamy,MD(S).,for the evaluation of Anti-Cancerous,Anti-Oxidant and Anti-Inflammatory activities.

The review literature reveals that the ganthagam and *Odukkan* have anti-tumour, anti-oxidant and anti-inflammatory activites.

Aim of the dissertation is to study the Anti-cancer,Anti-oxidant and Anti-inflammatory activities.

The test drug was prepared properly by given procedure. All the ingredients were identified and authenticated by expert of department of gunapadam.

The preparation of trial drug was standardized primarily by physicochemical analysis of the drug shows it is grey in colour with bitter astringent taste and odour.

As per the siddha literature diseases are caused due to change in derangement of three vital humours, the vital humour kabha is deranged and accompanied with ushna causes yoni puttru. The bitter taste normalized ushnam and kapham.

In physicochemical analysis, the water soluble ash value of the test drug is7.75% and acid soluble ash value is 0.95%.The loss on drying of the drug is7.30%.

The biochemical analysis shows the presence of sulphate and chloride. These content are having important role in physiological functions of the body. Microbial limit test shows it is free from microbial contamination.

The phytochemical analysis reveals the presence of alkaloid, tannins, carboxylates and glycosides, Alkaloids are having stimulant activities which controls the fatigue and lethargy of the cancer patients. Tannins having astringent, haemostatic, anti-septic and toxic properties, which control symptoms of cervical cancer.

In instrumental analysis the ICP-OES result shows the toxic heavy metals such as Al, As, Cd, Cu Ni and Pb are in below detection limit (BDL). It is evident that the safety of siddha medicine has been proved by the modern scientific way.

The FTIR shows the presence of Alkane, Carbon di oxide, Bromide, Iodide

SEM analysis of *GANDHAGA PARPAM* shows that the uniform distribution of particle present size is 20-40 which increase the efficacy and bioavailability of test drug. So very minimal quantity of medicine is enough to treat the disease.

Anti-cancerous activity study was carried out for *GANDHAGA PARPAM* by Dalton's Lymphoma ascites (DLA) cell was supplied by Amala cancer research center, Trissur, Kerala, India. The cells maintained in vivo in Swiss albino mice by intraperitoneal transplantation. While transforming the tumor cells to the grouped animal the DLA cells were aspirated from peritoneal cavity of the mice using saline. The cell counts were done and further dilution were made so that total cell should be 1 x 10^6, this dilution was given intraperitonealy. Let the tumor grow in the mice for minimum seven days before starting treatments. The result shows that *GANDHAGA PARPAM* 200mg/kg in DLA tumor bearing mice. The GP at the dose of 200mg/kg significantly inhibited the tumour volume, PCV, Tumour viable cell count and brought back the haematological parameter to more or less normal level.

The result showed that there was a concentration dependant anti-oxidant activity of *GANDHAGA PARPAM*. At the concentration [1.25-20µgm/ml] Percentage of inhibition increased from 9.8 to 88%. At the concentration of 20 µgm/ml there was an increased percentage of inhibition(88%) is scavenging the free radicals(DPPH).

The IC 50 value was obtained at 10µgm/ml.It is showed that *GANDHAGA PARPAM* is having significant anti oxidant activity.

Microbiology analysis result shows that GP controls bacterial cause of Chronic non healing cervical ulcer which is one of the causative factor of cancer.

The acute toxicity study shows that *GANDHAGA PARPAM* did not produce any toxic effect at dose of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg, and 2000mg/kg .

In sub acute toxicity test drug *GANDHAGA PARPAM* is did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable changes in hematological and biochemical parameter (50 to 200mg/kg body weight) over a period of 28 days. our result have demonstrated that the *GANDHAGA PARPAM* is relatively safe when administrated orally in rats.

It has been conducted that the *GANDHAGA PARPAM* is very effective in treating yoni putru(cervical cancer) without causing any adverse effects.

8. CONCLUSION

The trial drug *GANDHAGA PARPAM* is a herbo mineral, selected from the text book of *Agathiyar Vaithiya Vallathy - 600, P.No. 218*, 219 authored by Dr.A.Velusamy, MD.(s)., for Anti-cancerous, Anti-oxidant and Anti-inflammatory Activities and the results supported the study.

From the literature review, physico-chemical analysis, Bio-Chemical analysis, Pharamacological studies, Microbiological analysis, Instrumental analysis and toxicological studies it is concluded that the test drug of GP is safe and effective for all type of cancer (Yoni Puttru) and in safer to continue even for a long duration.

9. FUTURE SCOPE

Preclinical evaluation of the test drug *GANDHAGA PARPAM* has been done by bio-chemical, physio-chemical, instrumental, pharmacological, toxicological and microbial standard prescribed procedures. In future the drug has to validated by extensive clinical trials as per WHO guidelines. This *GANDHAGA PARPAM* is to be used very much to treating all types of cancer.

Having made up of nano particles, *GANDHAGA PARPAM* holds extra ordinary promise for the presentation and treatment of cancer. Thus the ancient wisdom siddhars will remains as one important source of future medicine and therapeutics

10. BIBLIOGRAPHY

1. Agathiyar Vaithiya Vallathy 600, Second Edition, Ayurvedha Siddha Vaithiya Araichi Kalagam, New Delhi – 110058. 2005.
2. Agathiyar 2000 3rd part, Saraswathy Mahal Noolagam, Thanjavur
3. Dr.R.Thiyagarajan.L.I.M, Gunapadam Thathu-Jeeva Vagupu, Published by Directorate of Indian Medicine and Homeopathy, Chennai, 2nd– 3rd part
4. Dr.C.S.Murugesa Muthaliyar, Siddha Material Medica (medicinal plant division), Published by Directorate of Indian medicine and homeopathy, Chennai, First edition; 2008, Pg. 162, 236, 490.
5. Rashtra Vardhana, Medicinal Plants of the World, Sarup Book Publishers, Pvt., Ltd.,
6. T.V. Sambasivampillai, Dictionary based on Indian Medicine science, published by Directorate of Indian Medicine and Homeopathy, Chennai, India.
7. Freshney RI. Culture of animal cells: A manual of basic technique. 5th edition, NY, Wiley-Liss, Inc., 1987.
8. Dr. R. Thiyagarajan, Siddha maruthuvam - Sirappu, published by Directorate of Indian Medicine and Homeopathy, Chennai.
9. Dr. K.S. Utthamnarayan, Aruvai Maruthuvam, published by Directorate of Indian Medicine and Homeopathy, Chennai.
10. Dr.S.Somasundaram, Maruthuva Thavaraviyal, Part 1, published by Elangovan pathipagam, Tirunelveli,.
11. Dr.S.Somasundaram, Angiospermkalin Vagaipadu, published by Elangovan pathipagam, Tirunelveli.
12. R. Alagappan, Manual of Practical Medicine, published by Fifth edition, Jaypee Brothers Medical Publishers (P) LTD, New delhi, Pg. 882.
13. K.N. Kuppusamy muthaliar, Siddha Vaithya Thirattu, published by Directorate of Indian Medicine and Homeopathy, Chennai.
14. Medicinal Plants used in Ayurveda and siddha, Volume 5, Published by Central council for Research in Ayurveda and Siddha, New delhi.
15. Dr. P.R. Ramasamy, Siddha Maruthuvathil Noi Neekuym Kanthagam.
16. Dr.K.M. Nadkarni's, Indian Materia Medica, Published by Popular prakashan private ltd., Mumbai.

17. K.R. Kirtikar, Indian medicinal plants, Volume 4, Bishen Mahendra Pal Singh, India.
18. C.Kannusami pillai, Sikicha Ratna Deepam Enum Vaidya Nool, – Published in 1991, Thirumagal Printers, Chennai.
19. Dr.M.Shanmugavel, Siddha Maruthuva Noi Naadal Noi Muthal Naadal Thirattu, Published by Directorate of Indian Medicine and Homeopathy, Chennai,
20. S.P.Ramachandran, Agathiyar Yemmathathuvam Ennum Panchakaviya Nigandu –Mahalakshmi Offset, Chennai.
21. Dr.V.Narayanaswami, Pharmacopia of Hospital of Indian medicine, 1995, 2nd edition, Govt branch press, Madurai.
22. A.K.Gupta, Wealth of India, vol-IV, Publication and Information Directorate, New Delhi, 1988 edition.
23. Dr.Arangarajan, Dr.prema, Theraiyar Gunavagadam, 2006 edition, saraswathi mahal library, Chennai.
24. J.S.Gamble, Flora of presidency of Madras, Vol-III, Shiva offset press, Dehradun.
25. www.wikipedia.com
26. DS.P.Ramachandran, Bogar Nigandu 1200, 1999 edition, thamarai noolagam, creative offset, Chennai.
27. Dr.C.Arangarasan, Agathiyar Attavanai vagadam, Aug 1991 edition, saraswathi mahal noolagam, velan press, Chidambaram.
28. Hakkim, Mugamed Abdullah shahib, Anuboga vaidya Navaneedam, 9th part, 2002 edition, Thamarai noolagam, creative offset, Chennai. Pg. 80-98.
29. A.K.Gupta, Madhu sharma, Indian Medicinal plants, vol-V, 2007 edition, Mehta offset, New Delhi.

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